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Influence of Experimental Pulmonary Emphysema on Toxicological Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust

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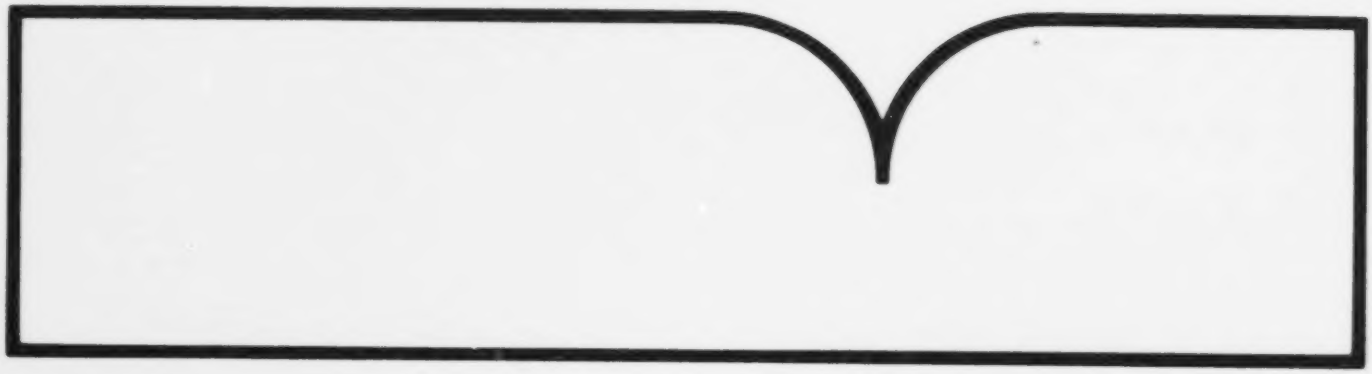

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15. Supplementary Notes

The hypothesis tested in this project was that rats with preexisting experimentally-induced pulmonary emphysema were more susceptible than rats with normal lungs to the adverse effects of exposure to NO_2 or diesel exhaust.

Rats were exposed by inhalation seven hr/day, five day/wk, for 24 months to NO_2 at 9.5 ppm, or to diesel exhaust at 3.5 mg soot/m^3, or to clean air. Pulmonary emphysema was induced in one-half of the rats by intratracheal instillation of elastase, six weeks before exposures. Nonneoplastic endpoints were evaluated after 12, 18, and 24 months of exposure.

Nitrogen dioxide exposure of normal rats caused mild epithelial hyperplasia and inflammation in proximal alveoli. Significant interactions between the influences of emphysema and nitrogen dioxide were demonstrated to be additive for four parameters (out of 61 parameters). Diesel-exhaust exposure of normal rats caused progressive, focal inflammation, and epithelial proliferation. The final soot lung burden was only one-third of that in nonemphysematous lungs. The effects of exhaust exposure in emphysematous rats were similar to, but less than, those in nonemphysematous rats, interactions between the influences of emphysema and exhaust were demonstrated for 19 parameters (out of 65 parameters).

In conclusion, emphysematous rats do not have increased susceptibility to NO_2 or exhaust, however, do have more abnormality than the rats with emphysema alone.

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Joe L. Mauderly, David E. Bice, Yung S. Cheng, Nancy A. Gillett, Rogene F. Henderson, John A. Pickrell, Ronald K. Wolff

Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM

Includes the Commentary by the Institute's Health Review Committee

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Influence of Experimental Pulmonary Emphysema on the Toxicological Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust

Joe L. Mauderly,[1] David E. Bice, Yung S. Cheng, Nancy A. Gillett, Rogene F. Henderson, John A. Pickrell, Ronald K. Wolff

ABSTRACT

This project examined the influence of preexisting, experimentally induced pulmonary emphysema on the adverse health effects in rats of chronic inhalation exposure to either nitrogen dioxide or automotive diesel-engine exhaust. Previous reports indicated that humans with chronic lung disease were among those most severely affected by episodic exposures to high concentrations of airborne toxicants. There were no previous reports comparing the effects of chronic inhalation exposure to components of automotive emissions in emphysematous and normal animals. The hypothesis tested in this project was that rats with preexisting pulmonary emphysema were more susceptible than rats with normal lungs to the adverse effects of the toxicant exposures.

Young adult rats were housed continuously in inhalation exposure chambers and exposed seven hours per day, five days per week, for 24 months to nitrogen dioxide at 9.5 parts per million (ppm)[2], or to diesel exhaust at 3.5 mg soot/m^3, or to clean air as control animals. These concentrations were selected to produce mild, but distinct, effects in rats with normal lungs. Pulmonary emphysema was induced in one-half of the rats by intratracheal instillation of the proteolytic enzyme elastase six weeks before the toxicant exposures began. Health effects were evaluated after 12, 18, and 24 months of exposure. The measurements included respiratory function, clearance of inhaled radiolabeled particles, pulmonary immune responses to instilled antigen, biochemistry and cytology of airway fluid, total lung collagen, histopathology, lung morphometry, and lung burdens of diesel soot. The significance of influences of emphysema and toxicant exposure, and interactions between influences of the two treatments, were evaluated by analysis of variance.

The elastase treatment resulted in pulmonary emphysema that was manifested by enlarged alveoli and alveolar ducts, and by ruptured alveolar septa. There was no accompanying inflammation and no alterations of bronchioles. The emphysema persisted throughout the study period, with little evidence of progression. Lung weight was increased, physiological lung volumes were enlarged, lung compliance was increased, and airflow was obstructed.

Nitrogen dioxide exposure of normal rats caused mild epithelial hyperplasia and a thickening of the walls of terminal bronchioles, an extension of bronchiolar epithelium into proximal alveoli, and inflammation in proximal alveoli. Lung volume and weight and the lung collagen content were increased. Airway fluid indicators of cell damage and oxidant protective mechanisms were increased. Similar effects of nitrogen dioxide exposure were superimposed over the effects of emphysema in emphysematous nitrogen dioxide-exposed rats. Several parameters were affected similarly by nitrogen dioxide exposure and emphysema (for example, increased lung volume), and the combined effects tended to be additive. Significant interactions between the influences of emphysema and nitrogen dioxide were demonstrated for four parameters, two indices of forced airflow limitation and two indices of airway fluid proteolytic activity. Only one parameter, an index of flow limitation, indicated that the influences of emphysema and nitrogen dioxide were more-than-additive, and this finding was not supported by other flow indices.

Diesel-exhaust exposure of normal rats caused progressive focal inflammation, epithelial proliferation, and fibrosis surrounding foci of aggregated soot-laden macrophages in alveoli. The lungs were smaller, stiffer, and heavier. Airway indicators of cytotoxicity, proteolytic activity, and turnover of lung collagen were increased, as was total lung collagen. The number of cells in pulmonary lymph nodes was increased, but response to antigen was not significantly altered. Soot accumulated less rapidly in emphysematous than in nonemphysematous lungs, resulting in a final soot lung burden only one-third of that in nonemphysematous lungs. The effects of exhaust exposure in emphysematous rats were qualitatively similar to those in nonemphysematous rats. The magnitudes of the effects in emphysematous rats were less, however, in parallel to their smaller soot lung burden. Significant interactions between the influences of emphysema and exhaust were demonstrated for 19 parameters, including indices of respiratory function, airway fluid, particle clearance, lung collagen, lung weight, and body weight. Only one parameter, the body weight of small

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[2] A list of abbreviations appears at the end of this report for your reference.

groups of rats killed for morphological evaluation, indicated that the influences of emphysema and exhaust exposure were more-than-additive, and this finding was not supported by body-weight data from larger groups of rats used for respiratory function tests.

These results were not consistent with the hypothesis that emphysematous rats have increased susceptibility to nitrogen dioxide or exhaust. This finding suggests that individuals with pulmonary emphysema might not have increased susceptibility to chronic inhalation exposures to these materials. Overall, however, there was more evidence of abnormality in the toxicant-exposed emphysematous rats than in the rats with emphysema alone. This finding suggests that the superimposition of exposure-related effects over preexisting chronic lung disease might justify special concern for such individuals.

INTRODUCTION

Concern for human subpopulations with potentially increased susceptibility to the effects of inhaled toxic materials is mandated by the Clean Air Act (1983), which legislates protection of the public health from airborne pollutants and has been interpreted to include protection of "any group of the population" (U.S. Senate 1970). Preexisting chronic lung disease is among the factors thought likely to be related to increased susceptibility. The Senate Committee on Public Works specified that the populations of concern in the Clean Air Act included "particularly sensitive citizens such as bronchial asthmatics and emphysematics" (U.S. Senate 1970). The research reported herein was conducted under sponsorship of the Health Effects Institute, to examine the possibility that individuals with chronic lung disease constitute a subpopulation especially susceptible to inhaled environmental pollutants associated with automotive emissions.

Epidemiological data suggest that airborne pollutants have greater effects on humans with diseased lungs than on normal subjects. Increases in mortality and morbidity from respiratory disorders were documented to have occurred primarily in subjects with preexisting lung disease during episodic, severe increases in pollution levels in the Meuse Valley, Belgium; Donora, PA; London, England; New York, NY; Osaka, Japan (Higgins and Ferris 1973); Los Angeles, CA (Motley 1971); Chicago, IL (Carnow et al. 1969); and the Netherlands (Van der Lende et al. 1975). Patterns of morbidity among subjects with chronic lung disorders have also been positively correlated with less abrupt fluctuations in airborne particles and gases. People with asthma appear particularly susceptible to increases in sulfur oxides and particulate sulfates (Motley 1971; French 1975; U.S. Environmental Protection Agency 1975; Kahn 1977; Kinsman et al. 1981), although they appear to have little increased susceptibility to oxidants. Subjects with chronic obstructive lung disease (emphysema and chronic bronchitis) may also have increased susceptibility to inhaled pollutants (Motley 1971; Bruderman 1976; Zagranski et al. 1979). Most of the above information is derived from observations during acute, severe pollution episodes. Human subjects with chronic lung disease have also been experimentally exposed to pollutants for short periods to study acute respiratory function responses. Little is known, however, about the relative susceptibilities of normal humans and humans with preexisting chronic lung disease to the adverse effects of chronic inhalation exposures to materials in automotive emissions.

The most prevalent nonneoplastic, irreversible group of chronic lung diseases is the chronic obstructive lung disease complex, consisting primarily of chronic bronchitis, emphysema, or combinations of both (Bates et al. 1971). In 1973, chronic bronchitis was estimated to affect 10 to 25 percent of the adult U.S. population (Wilson 1973), and in 1977, pulmonary emphysema was estimated to be the cause of death of approximately 2 percent of the adult U.S. population (Rockette 1977). Information on the effects of these diseases on susceptibility to inhaled toxicants clearly would be useful for estimating health risks from automotive emissions and for setting standards for levels of airborne toxicants.

Work with animal models of human chronic obstructive lung disease has focused primarily on emphysema. Chronic bronchitis is difficult to model in laboratory animals because mucous hypersecretion is a major feature of the disease in humans, and the mucous secretory elements in airways of most laboratory animals differ greatly from those of humans. Inhalation exposure to a high concentration of sulfur dioxide has been used most frequently to cause chronic bronchitis in animals (Greene et al. 1984). This model is not satisfactory for studying the effects of chronic inhalation of automotive emissions on bronchitic lungs because the lesions regress after cessation of exposure to sulfur dioxide; thus, continuous exposure is required to maintain the lesions. Pulmonary emphysema, however, is readily induced by a single intratracheal instillation of proteolytic enzyme, most commonly elastase, and persists without further treatment for the remainder of the animal's life. There is a considerable body of literature on enzyme-induced emphysema in laboratory animals. This information was thoroughly reviewed by Snider and coworkers (1986). Instillation of the enzyme causes an intense destructive and inflammatory response that resolves and results in a permanent enlargement of alveoli and alveolar ducts,

with alveolar septal disruption but no accompanying inflammation. The resulting emphysema does not closely model the progressive emphysema of human smokers, with its accompanying alveolar inflammation and bronchitis. The lesion most closely resembles the uncomplicated, panacinar emphysema occurring in humans with antiproteinase deficiency. Although a model of continuously evolving emphysema accompanied by alveolar and bronchiolar inflammation would be desirable, the enzyme-induced emphysema provides an opportunity to examine the susceptibility of lungs with stable emphysematous lesions to inhaled toxicants.

There have been no studies comparing the susceptibilities of normal animals and animals with pulmonary emphysema to the effects of long-term inhalation exposures to components of automotive emissions. Only one study has involved repeated exposures to inhaled toxicants and observations for as long as one year. Gross and colleagues (Gross and deTreville 1969; Gross et al. 1971) exposed normal and emphysematous rats and hamsters by inhalation for 115 days over a one-year period to high concentrations of quartz or coal dust. At the end of exposure, the lungs of the emphysematous animals contained less of both dusts than the lungs of the normal animals. Lung weights of all groups were measured, and collagen and lipid contents of the quartz-exposed animals were measured. The findings were mixed with regard to the relationship between emphysema and health effects, with some parameters indicating reduced responses in emphysematous animals and some indicating enhanced responses.

There have been several short-term studies of emphysematous animals exposed to inhaled toxicants. Goldring and associates (1970) reported that papain-induced emphysema enhanced the inflammatory response of hamsters to a three-month exposure to a high concentration of sulfur dioxide, but exposure-related changes in respiratory function were not significantly different. Niewoehner and Kleinerman (1973) reported that the effects of papain-induced emphysema and exposure to a high concentration of nitrogen dioxide (NO_2) for up to 10 days tended to be additive, but there was little difference in lung structure or function between the groups. Raub and coworkers (1983) reported that elastase-induced emphysema slightly enhanced the histopathological and respiratory functional effects of a 28-day exposure of hamsters to a reaction mixture of olefin, ozone, and sulfur dioxide. Busch and colleagues (1984) reported that elastase-induced emphysema had little effect on the responses of rats and guinea pigs exposed for four weeks to ammonium sulfate particles. Kimmel and coworkers (1985) and Lai and Diamond (1986) exposed rats for 12 weeks to cigarette smoke, starting three days after elastase instillation, and reported that smoke exposure enhanced the development of emphysema.

The studies described above present an uncertain view of the relative susceptibilities of normal and emphysematous lungs to inhaled toxicants. In addition, none addresses the issue of long-term exposure to automotive emissions. The present study was designed to compare the susceptibilities of normal and emphysematous lungs to chronically inhaled automotive emissions. Emphysema induced by intratracheally instilled elastase was used because of the considerable previous experience with this model at this Institute (Lundgren et al. 1981; Harkema et al. 1982, 1984; Likens and Mauderly 1982; Damon et al. 1983; Mauderly 1984b). Two forms of automotive emissions were selected. Nitrogen dioxide was chosen as a single exposure material, since it is an oxidant gas present in fresh automotive exhaust, and diesel exhaust was chosen as a complex automotive emissions atmosphere containing gases, vapors, and particles.

SPECIFIC AIMS

This project examined the influence of pulmonary emphysema on the responses of rats to chronic inhalation exposure to either NO_2 or diesel exhaust. The aim was to estimate whether or not humans with emphysema might be a population with increased susceptibility to inhaled automotive emissions. The objective was to compare the effects of the toxicant exposures in rats with preexisting pulmonary emphysema to those in rats with normal lungs. The hypothesis tested was that the exposure-related effects in the emphysematous rats would be greater than (or different from) those in nonemphysematous rats. The project was initiated in response to information needs stated by the Health Effects Institute in Request for Applications 82–3, "Models of Susceptible Populations."

The goals of this project were to expose emphysematous and normal rats to NO_2 or diesel exhaust repeatedly for a major portion of their life span, to measure health effects parameters serially during the exposure, and to evaluate the data for interactions between the influences of emphysema and toxicant exposure. Single exposure concentrations of NO_2 and diesel exhaust were employed; evaluation of dose-response relationships was not a goal of this project. The choice of concentration presented a conflict between the need for information related to current ambient exposure levels and the need to induce measurable adverse health effects. Concentrations sufficient to cause mild, but distinct, effects in normal rats were chosen. The concentrations required to achieve these effects were much higher than current ambient levels.

METHODS

EXPERIMENTAL DESIGN

Male Fischer-344 rats were exposed by inhalation for 24 months to NO_2, to diluted automotive diesel exhaust, or to clean air as sham-exposed controls, beginning at 18 weeks of age. At six weeks before exposure, one-half of the rats from each exposure group were instilled intratracheally with porcine pancreatic elastase to induce pulmonary emphysema. The resulting six experimental groups were designated as follows with respect to elastase treatment and exposure: untreated, sham-exposed, normal control rats (C); elastase-treated, sham-exposed, emphysematous control rats (E); untreated, NO_2-exposed rats (N); elastase-treated, emphysematous, NO_2-exposed rats (E + N); untreated, diesel-exhaust-exposed rats (D); and elastase-treated, emphysematous, diesel-exhaust-exposed rats (E + D). The experimental groups and numbers of rats entering chronic exposure are outlined in Table 1.

All rats were housed continuously in inhalation exposure chambers and were exposed to the experimental atmospheres seven hours per day, five days per week. Nominal concentrations of 9.5 ppm NO_2 or diesel exhaust at 3.5 mg soot/m^3 were used. These concentrations were selected on the basis of previous results, from this Institute, of mild health effects without mortality among rats exposed to NO_2 (Pickrell et al. 1981; Gregory et al. 1983; Behr et al. 1984) or diesel exhaust (Bice et al. 1985; Mauderly et al. 1987a, 1988; Wolff et al. 1987).

The preexposure characteristics of the emphysema were evaluated by measuring respiratory function and examining the lung histopathology of eight elastase-treated and eight untreated rats two weeks before chronic exposures began. Health effects among the rats exposed chronically were evaluated in detail after 12, 18, and 24 months of exposure. These evaluations and the allocation of rats from each experimental group are outlined in Table 2. The rationale for selecting the health effects evaluations is presented in Appendix A. Functional evaluations included respiratory function, immune responses in pulmonary lymph nodes, and clearance of radiolabeled particles from the lung. Evaluations of biochemical and structural lung damage included cellular, enzyme, and protein composition of airway fluid, total lung collagen content, excised lung weight and fixed lung volume, terminal airspace diameter, and qualitative histopathology. The amounts of diesel soot in the lungs of exhaust-exposed rats were also measured.

Immune responses were measured only in control and exhaust-exposed rats after 24 months of exposure, because our interest was in whether or not the translocation of soot particles to pulmonary lymph nodes would have any effect on the immune responses of lymph node cells. The total lung collagen of all groups was measured once, after 24 months of exposure. Hydroxyproline-containing collagenous peptides in airway fluid were measured after 12, 18, and 24 months of exposure to describe the time course of the turnover of the lung extracellular collagen matrix. Rats from all groups were exposed to radiolabeled particles once, after 18 months of exposure to the experimental atmospheres, and clearance of these particles was measured during the final six months of experimental exposure.

Table 1. Experimental Groups of Male Fischer-344 Rats Used to Compare Effects of Inhaled Automotive Emissions on Normal and Emphysematous Lungs

	Number of Rats		
Exposure Group	Normal	Emphysematous	Total
Nitrogen dioxide (9.5 ppm)	46 (N)	46 (E + N)	92
Diesel exhaust (3.5 mg soot/m^3)	46 (D)	46 (E + D)	92
Sham-exposed control	46 (C)	46 (E)	92
Total	138	138	276

Table 2. Allocation of Each Group of 46 Rats for Evaluations of Health Effects

	Number of Rats	
Allocation	Evaluated	Removed by Death or Killing
12 Months of Exposure		
Respiratory function	16	
Combined sacrifice[a]	8	8
18 Months of Exposure		
Respiratory function	16	
Clearance of radiolabeled particles	10	
Combined sacrifice[a]	8	8
24 Months of Exposure		
Respiratory function	16	
Pulmonary immune responses	8	8
Combined sacrifice[a]	8	8
Subtotal		32
Allowance for mortality		14
Total		46

[a] Killed for airway fluid assays, lung burdens of soot, and histopathology. Measurements of lung collagen were also included at 24 months.

The above evaluations required a total of 32 rats per group, as shown in Table 2. An additional 14 rats were included in each group to allow for mortality, resulting either from experimental procedures or from natural causes. On the basis of results from previous long-term diesel-exhaust exposures (Mauderly et al. 1987a), it was expected that the exposures would not affect survival, and that approximately 60 percent of the rats entered into the study (and not killed) would survive to the end of exposure.

PROCEDURES

Animals and Maintenance

Male Fischer-344/Crl rats were obtained from the Institute's specific pathogen-free breeding colony, which was derived from stock obtained from the National Institutes of Health. The rats were randomized by litter, and those designated for the emphysematous groups were instilled with elastase at 12 weeks of age. All rats were moved to inhalation exposure chambers at 15 weeks of age for preexposure acclimatization. Rats for preexposure evaluation of emphysema were removed, tested, and killed one week later, at 16 weeks of age. The exposures began two weeks later, when the rats were 18 ± 1 weeks of age. At the end of exposures, 24 months later, the rats were 122 weeks (854 days) of age.

All rats were housed in barrier-maintained colony housing until acclimatization in exposure chambers began. They were housed two per polycarbonate cage, with filter tops and sterilized hardwood-chip bedding. Feed (Wayne Lab Blox, Allied Mills, Chicago, IL) and water were provided ad libitum. Rooms were maintained at 20° to 22°C, with a relative humidity of 40 to 60 percent, and a 12-hour light-dark cycle (light 0600 to 1800).

From the beginning of acclimatization until the end of the study, the rats were housed continuously in wire cages, within glass and stainless steel chambers having a volume of 2 m^3 (H-2000, Hazleton Systems, Aberdeen, MD). Water was provided ad libitum, and feed was available outside of exposure hours. Chambers were maintained at 25° to 29°C, 40 to 60 percent relative humidity, 15-cfm airflow, and on a 12-hour light-dark cycle (light 0600 to 1800). Bacteriostatic cageboard in excreta trays was changed twice daily, trays were washed daily, and chambers were washed weekly.

It was important to determine that the rats were free from pulmonary microbial infections. Serum was collected from two rats in each experimental group killed after 12 and 24 months of exposure, and was submitted to an independent laboratory (Microbiological Associates, Bethesda, MD) for analysis of antibody titers to *Mycoplasma pulmonis* and nine pathogenic viruses.

Induction of Emphysema

Emphysema was induced by intratracheal instillation of porcine pancreatic elastase, using procedures found suitable in previous studies at this Institute (Harkema et al. 1982, 1984; Likens and Mauderly 1982; Mauderly 1984b). Elastase was obtained as lyophilized powder (Catalog No. 324689, Lot No. 203006, 108 units/mg, Calbiochem-Behring, La Jolla, CA), and kept frozen until use.

The rats to be instilled were selected by computer-generated random numbers and weighed. A stock solution was made of elastase in saline, at a concentration yielding 0.5 unit of elastase per gram of body weight, in a total of 1.0 ml for the heaviest rat. An appropriate amount of this stock solution, based on the weight of each rat, was drawn into a syringe, and the total volume was adjusted to 1.0 ml for each rat by adding saline.

Each rat was anesthetized with halothane in oxygen, intubated with an orotracheal catheter, and placed prone on an intubation platform, as described in detail previously (Mauderly 1977). Placement of the catheter in the trachea was confirmed by observing the breathing-induced movement of the plunger of a smooth glass syringe that was attached to the catheter. The rat was then given a few deep breaths of air from a syringe, to induce temporary apnea. The elastase-saline solution was then instilled from a syringe via a smaller catheter passed through the orotracheal catheter. The tip of the orotracheal catheter reached mid-trachea, and the smaller catheter extended approximately 2 mm beyond; thus, the elastase solution was deposited just above the tracheal bifurcation. The small catheter was then withdrawn and a syringe was used to inflate the lung with a few milliliters of air, to push the solution into the lung, and to reestablish ventilation. As soon as the rat resumed breathing, it was extubated and placed in a plastic cage for recovery from anesthesia.

Exposures and Measurement of Exposure Atmospheres

Rats were exposed seven hours per day (0800 to 1500), five days per week, for 24 months to NO_2 diluted to 9.5 ppm, or to whole diesel exhaust diluted to a soot concentration of 3.5 mg/m^3, or to filtered air. Details of the generation and measurement of exposure atmospheres are presented in Appendix B. Concentrations of key constituents of the exposure atmospheres are summarized in Table 3.

Nitrogen dioxide was generated by controlled vaporization of liquid nitrogen tetroxide dimer (Matheson, East Rutherford, NJ) in a stream of dry nitrogen (N_2), and diluted with filtered air to the desired concentration. The concentration of NO_2 in the chamber was monitored continuously by chemiluminescent analysis. The concentrations were periodically cross-checked by wet chemistry.

Table 3. Summary of Concentrations of Key Constituents of Exposure Atmospheres During the 24 Months of Exposure

Exposure Atmosphere	Unit	Mean[a]	SE
Nitrogen dioxide	ppm	9.5	0.1
Diesel exhaust			
Total particles	$\mu g/m^3$	3,490	29
Carbon monoxide	ppm	9.8	0.4
Carbon dioxide	ppm	2,740	60
Hydrocarbon vapors	ppm	3.1	0.2
Nitric oxide	ppm	3.0	0.2
Nitrogen dioxide	ppm	1.2	0.1
Ammonia	ppm	0.5	0.1
Sham-exposed air control[b]			
Total particles	$\mu g/m^3$	7.2	0.4
Carbon dioxide	ppm	1,360	40
Ammonia	ppm	0.3	0.02

[a] Mean ± SE of weekly mean values sampled at midpoints in the chambers.

[b] Materials generated by the rats' presence and presumed to be included in the other chamber atmospheres.

Exhaust exposures were conducted using methods previously described (Mokler et al. 1984). Exhaust was generated by 1980-model 5.7-liter Oldsmobile engines, burning fuel that met the U.S. Environmental Protection Agency (EPA) certification standards, mounted on test stands, and operated by computer on the Federal Test Procedure urban-duty cycle. The exhaust was passed through a standard automotive exhaust system, including muffler, diluted 1:10 with filtered air in a dilution tunnel, and then serially diluted to the final concentration. Characteristics of the exhaust atmosphere and methods of measurement were previously reported (Cheng et al. 1984). The concentration of soot particles in the chamber was measured gravimetrically by daily filter samples. Bag samples were taken weekly for analysis of gases and vapors, including carbon monoxide (CO), carbon dioxide, vapor-phase hydrocarbons, nitric oxide, NO_2, total N_2, and ammonia.

The chambers housing the sham-exposed rats were continuously ventilated with air that had passed through high-efficiency particulate air (HEPA) filters. Concentrations of particles, arising primarily from the animals themselves, were measured by daily filter samples; and weekly bag samples were analyzed for background gas and vapor concentrations.

Evaluation of Health Effects

The rats were observed twice daily for morbidity and mortality, and were weighed monthly. Other health effects among rats within each experimental group were evaluated according to the outline presented in Table 2.

The respiratory function of 16 rats per group was measured after 12, 18, and 24 months of exposure. Eight of these rats were measured serially (at all times), and at each time eight were killed for evaluation of airway fluids and morphology. Respiratory function was measured by plethysmography, as previously described (Harkema et al. 1982). Rats were anesthetized with halothane in air, intubated orally with tracheal and esophageal catheters, and placed prone in a 1.4-liter combination volume-displacement and constant-volume plethysmograph. Anesthetic depth was adjusted to yield a respiratory frequency between 50 and 60 breaths per minute, and for the induction of temporary apnea after two to three deep lung inflations. Respiratory patterns and dynamic lung mechanics were measured during spontaneous breathing. Single-breath tests, including lung volume subdivisions, quasistatic pressure-volume relationships, CO diffusing capacity, single-breath N_2 washout, and forced expiration, were performed by inducing apnea by inflation and applying positive and negative airway pressures. The lung volume at 30 cm water transpulmonary pressure was defined as total lung capacity. Forced expirations were induced using a negative airway pressure of 50 cm water. Quasistatic inflations and deflations were done using flow rates of 5 ml/sec and 3 ml/sec, respectively.

One week after the respiratory function tests, eight rats per group were weighed, anesthetized with halothane, and killed by cervical dislocation. A complete necropsy was performed, and tissues from major organs were fixed for potential histopathological evaluation. The heart-lung block was removed and weighed, the left bronchus was clamped with two hemostats, and the left and right lungs were separated between the hemostats. The left lung was used for morphological evaluations and the right lung was used for biochemistry and soot-lung-burden assays.

Airway fluids were assayed for cellular and biochemical indicators of toxicity, as described previously (Henderson et al. 1981, 1985; Henderson 1984). The right lung was lavaged with two 5-ml washes of saline. The recovered fluid was pooled, cells were removed by centrifugation, and both total and differential cell counts were performed. The supernatant was analyzed for cytoplasmic, lysosomal, and proteolytic enzymes, total protein, and hydroxyproline-containing collagenous peptides (Pickrell et al. 1981).

The right lung was then perfused intravascularly with saline to remove blood, the heart was removed, and the lung was homogenized in Tris buffer. The amount of soot in the lungs of exhaust-exposed rats was estimated, as described previously (Henderson et al. 1987), by comparing absorption of light at 620 nm by the homogenate to that of

homogenates of unexposed lungs spiked with known quantities of soot. Previous work (Henderson et al. 1982) had demonstrated that lavage removed less than 1 percent of the diesel soot from the lungs of rats exposed chronically. The homogenates from rats killed at 24 months were also analyzed for total lung tissue collagen by assaying for hydroxyproline after acid hydrolysis of the lung tissue (Grant 1964).

A cannula was placed in the left bronchus, and the left lung was suspended by cannula in 10 percent neutral buffered formalin and fixed for 24 hours by constant airway perfusion at a pressure of 20 cm fixative. The external volume of the fixed lung was then measured by water displacement. A multiple-blade device was used to section the entire left lung into 3-mm-thick slices, which were examined grossly for lesions, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Ten to fifteen of these sections per animal were used for both qualitative histopathology and for morphometry. A total of 24 fields for each rat (5 to 6 per section) were examined under a grid to calculate the mean linear intercept of terminal airspaces (alveoli and alveolar ducts) (Dunnill 1962). Pulmonary lymph nodes were also sectioned at 5 µm, embedded in paraffin, stained with hematoxylin and eosin, and examined for histopathology.

Pulmonary immune responses of the control and exhaust-exposed rats were evaluated after 24 months of exposure, as previously described (Bice et al. 1979, 1985; Bice and Schnizlein 1980). One week before scheduled killing, the rats were immunized by intratracheal instillation of 10^8 sheep red blood cells in 0.3 ml saline. The rats were killed by halothane anesthesia, exsanguination, and cervical dislocation. The tracheobronchial and parathymic lymph nodes were removed, and cell suspensions were made from the nodes. The total number of cells in these lymph nodes was determined using a Coulter counter, and the number of cells producing IgM antibody to the sheep red blood cells was determined by the Cunningham modification of the Jerne plaque assay. Serum samples were analyzed for IgM antibody specific to sheep red blood cells using an enzyme-linked immunosorbent assay.

Rats from each experimental group were exposed to radiolabeled particles for measurement of lung clearance efficiency after 18 months of NO_2 or exhaust exposure. The rats then continued to be exposed to NO_2 or exhaust and were killed after 24 months of exposure. The rats received a single, brief, nose-only inhalation exposure to monodisperse ^{134}Cs-labeled fused aluminosilicate particles that had a mass median diameter of 1.0 µm and a geometric standard deviation of less than 1.2, as described previously (Snipes et al. 1983). These particles have very low solubility in the lung. The radiolabel remains primarily incorporated within the particles, and the small amount of ^{134}Cs that leaches from the particles is rapidly excreted. Therefore, clearance of the particles from the lung can be followed by serial measurements of whole-body radioactivity. Whole-body counts of ^{134}Cs activity (half-life = 2.05 years) were performed at 1, 4, 8, 16, 28, 56, 84, and 112 days after exposure to the labeled particles.

Statistical Analyses

The goal of this study was to determine whether or not rats with pulmonary emphysema were more susceptible than rats with normal lungs to the adverse effects of exposure to NO_2 or diesel exhaust. The primary statistical tool used to accomplish this goal was analysis of variance (ANOVA). The approach was based on a definition of increased susceptibility that included (1) a statistically significant interaction between the influences of emphysema and pollutant exposure; and (2) effects of emphysema and pollutant exposure that are more-than-additive at the final measurement, the time at which the effects would be greatest. The criterion for statistical significance was set at $p < 0.05$ (two-tailed) for all comparisons.

First, ANOVA was applied to the entire data base for each measured parameter to detect significant interactions between emphysema and pollutant exposure (either NO_2 or diesel exhaust). Three-way ANOVA was used for parameters measured serially (after 12, 18, and 24 months of exposure), to include all data in the examination of the significances of the influences of emphysema, exposure, and time, and the significances of the interactions among the three treatment variables. Two-way ANOVA was used for parameters measured only once, to examine the significances of the influences of emphysema and exposure, and the significances of the interactions between emphysema and exposure. The ANOVA results for all parameters measured in the study are presented in the appendices, and parameters with significant emphysema-exposure interactions are listed in tables in the following sections.

Second, parameters indicated by ANOVA to have significant interactions between emphysema and exposure were then examined to determine if the influences of emphysema and exposure were additive, more-than-additive, or less-than-additive. This was done by comparing the directions and magnitudes of the absolute differences between the mean values of each of the treated groups (E, N, E + N, or E, D, E + D) and the control group (C) at the last measurement (after 24 months of exposure for most parameters; between 18 and 24 months of exposure for particle clearance). If the difference between mean values of the emphysematous exposed group and the control group (E + N − C, or E + D −

C) was greater than the sum of the differences due to emphysema or exposure alone ([E – C] + [N – C], or [E – C] + [D – C]), the effect was termed "greater-than-additive." An interaction of this nature would suggest that rats with emphysematous lungs were more susceptible to the effects of exposure than were rats with normal lungs. If the sum of the differences due to emphysema and exposure alone was the same as the difference due to the combined treatment, the effect was termed "additive." An interaction of this nature would suggest that rats with emphysematous and normal lungs had similar susceptibilities to the effects of exposure. If the sum of the differences due to emphysema and exposure was less than the difference due to the combined treatment, the effect was termed "less-than-additive." An interaction of this nature would suggest that rats with emphysematous lungs were less susceptible to the effects of exposure than rats with normal lungs. These results are presented in tabular form in the following sections.

In addition to examining the issue of susceptibility, this study also provided considerable data on the nature and magnitude of the adverse health effects of emphysema, NO_2 exposure, and diesel-exhaust exposure. The two- and three-way ANOVA described above examined the influence of emphysema by pooling pollutant-exposed and unexposed groups, and the influence of exposure by pooling normal and emphysematous groups. To discriminate further between emphysema and exposure effects, multiple comparisons were used to determine the significance of differences among treatment groups at each measurement time. For each parameter, one-way ANOVA was first used to determine if there were any significant differences among treated and control groups at that time. Multiple two-tailed *t* tests were then used to test differences between specific groups, using the Games and Bonferroni methods to adjust critical *t* values for multiple contrasts. This approach was used to test differences between corresponding emphysematous and nonemphysematous groups (E versus C, E + N versus N, etc.) and between corresponding pollutant-exposed and unexposed groups (N versus C, E + N versus E, etc.). All of the data collected in this study were evaluated in the above manner, and the results are summarized in the appendices.

The progression of emphysema with time was examined by using two-way ANOVA to test the significance of interactions between the influences of emphysema and time on selected respiratory function and lung morphology parameters. These were measured on unexposed normal rats and emphysematous rats at base line and after 12, 18, and 24 months of exposure. To further illustrate the persistence of emphysema, selected base-line and 24-month values for emphysematous rats were expressed as percentages of the values for time-matched nonemphysematous rats. No statistical analyses were performed on the percentage-transformed data. These results are summarized in tabular form in the following section. Additional details on statistical methods are presented in Appendix C.

RESULTS

GENERAL FINDINGS

There were no significant effects of either emphysema or exposure on body weight, morbidity, or mortality. All rats appeared healthy throughout the study. The effects of both the emphysema and the exposures were sufficiently mild that the rats in all treatment groups appeared normal at all times, with the exception of discoloration of the haircoat by soot in the exhaust-exposed groups. All sera were negative for *Mycoplasma pulmonis* and pathogenic viruses at 12 and 24 months. One lung tumor was observed, a small bronchioloalveolar carcinoma in a nonemphysematous diesel-exhaust-exposed rat killed at 24 months.

The data for all parameters measured at 12, 18, and 24 months for respiratory function, airway fluid, lung collagen, lung morphometry, clearance of radiolabeled particles, lung burdens of diesel soot, and pulmonary immune responses are summarized in Appendices D, E, F, G, H, I, and J, respectively. The parameters significantly altered by emphysema, NO_2, or diesel-exhaust exposure at each measurement time are indicated. The appendices also contain results of two-way or three-way ANOVA, and indicate the significant influences of emphysema, NO_2 or exhaust exposure, and time, as well as the interactions among these influences for each parameter. Since three-way ANOVAs incorporated data from all measurement times, significant influences of the exposures were demonstrated for some parameters for which no significant differences between control and exposed rats existed at single measurement times.

In the following sections, the characteristics of the elastase-induced emphysema and its effects on the parameters measured are described first. The influence of emphysema on the effects of exposure to NO_2 and diesel exhaust are then described in turn. For each pollutant, the effects of exposure on rats without emphysema are described first, followed by a description of the influence of emphysema on response to exposure. Responses of rats with and without emphysema are compared for parameters for which ANOVA indicated a significant interaction between emphysema and exposure. The Discussion section is similarly organized.

EFFECT OF EMPHYSEMA ALONE

The desired mild degree of pulmonary emphysema was achieved by the instillation of 0.5 IU elastase per gram of body weight. There was no emphysema-related mortality after the first two days after instillation, and the clinical appearance of the E rats was indistinguishable from that of the C rats throughout the study. The presence of mild pulmonary emphysema was clearly demonstrated by the base-line respiratory function and morphological assays (Table 4). The lungs of E rats were larger at standard distending pressures, both in vivo (total lung capacity) and when excised. Relaxed lung volume (functional residual capacity) and lung compliance were increased, while alveolar-capillary gas exchange (CO diffusing capacity) and forced expiratory flow rates (mean midexpiratory flow) were reduced. These changes reflected the loss of elastic recoil and the resulting airflow obstruction typical of elastase-induced emphysema in rodents (Harkema et al. 1982, 1984). Lung weight was increased, but not in proportion to lung volume; thus, lung density (weight/volume), when compared with base line, was reduced. Alveolar and alveolar duct sizes (mean linear intercept) were increased.

Emphysema was clearly evident in lungs of elastase-treated rats at all sacrifice times, regardless of additional exposure. Airspace dilatation with ruptured alveolar septa, and with no accompanying inflammation, was observed in all lung lobes at base-line sacrifice. The microscopic appearances of the lung parenchyma of C and E rats at 24 months are compared in Figure 1. The C rats had no significant lung lesions at any time (see block C in Figure 1). A slight, age-related dilatation of terminal airspaces occurred, as reported previously (Mauderly et al. 1984a), reflected by the slight progressive increase in mean linear intercept of the normal controls (Appendix G). The lungs of E rats had panacinar emphysema distributed in a patchy manner in all lobes (see block E in Figure 1). The lesion was consistent at all sacrifice times, with little qualitative evidence of progression and only a slight time-related increase in mean linear intercept (Appendix G). No foci of inflammation or fibrosis were observed in lungs from E rats at any time.

The persistence of the emphysema was demonstrated by comparing the magnitudes of selected effects on respiratory function and lung morphometry at base line and after 24 months of sham exposure (Table 4). Emphysematous rats weighed slightly less than normal rats throughout the study, but the differences were never significant. Total lung capacity was significantly greater at 24 months than at base line. The significant interaction between the influences of emphysema and time, demonstrated by ANOVA using data from all treatment groups and measurement times, indicated a progression of the effect. The mean linear intercept of terminal airspaces was also significantly more affected by emphysema at 24 months than at base line, and also demonstrated a significant interaction between emphysema and time. The emphysema-related increase in functional residual capacity, expressed as a fraction of total lung capacity, was also significantly greater at 24 months, but the interac-

Table 4. Effects of Emphysema on Respiratory Function and Lung Structure Before and After Exposures[a]

	Emphysematous Control (E) as Percentages of Normal Control (C)				Significant Influences of Emphysema (E) and Time (T)[b]		
	Base Line (n = 8)		24 Months (n = 16)				
Parameter	Mean	SE	Mean	SE	E	T	E × T
Body weight	97	2	96	2		+	
Total lung capacity (TLC)	106	1	118	3	+	+	+
Functional residual capacity (FRC)/TLC	114	4	136	4	+	+	
Maximum quasistatic chord compliance	123	8	134	7	+	+	
CO diffusing capacity (DL_{CO})/lung volume	81	4	77	6	+	+	
Mean midexpiratory flow	74	3	67	3	+		
Excised, fixed lung volume	147	10	134	3	+	+	
Lung weight/body weight	114	2	118	3	+	+	
Lung weight/lung volume	76	6	84	8	+	+	
Mean linear intercept	162	2	181	4	+	+	+

[a] Values of emphysematous control rats (E) for selected parameters measured before NO_2 and diesel exhaust exposures were initiated (base line), and after 24 months of exposure (Tables A.3 and G.3), are expressed as mean (± SE) percentages of mean values for normal control rats (C).

[b] Two-way ANOVA was applied to data collected from emphysematous and normal control rats at base line, 12, 18, and 24 months to determine the significances of influences of emphysema and time.

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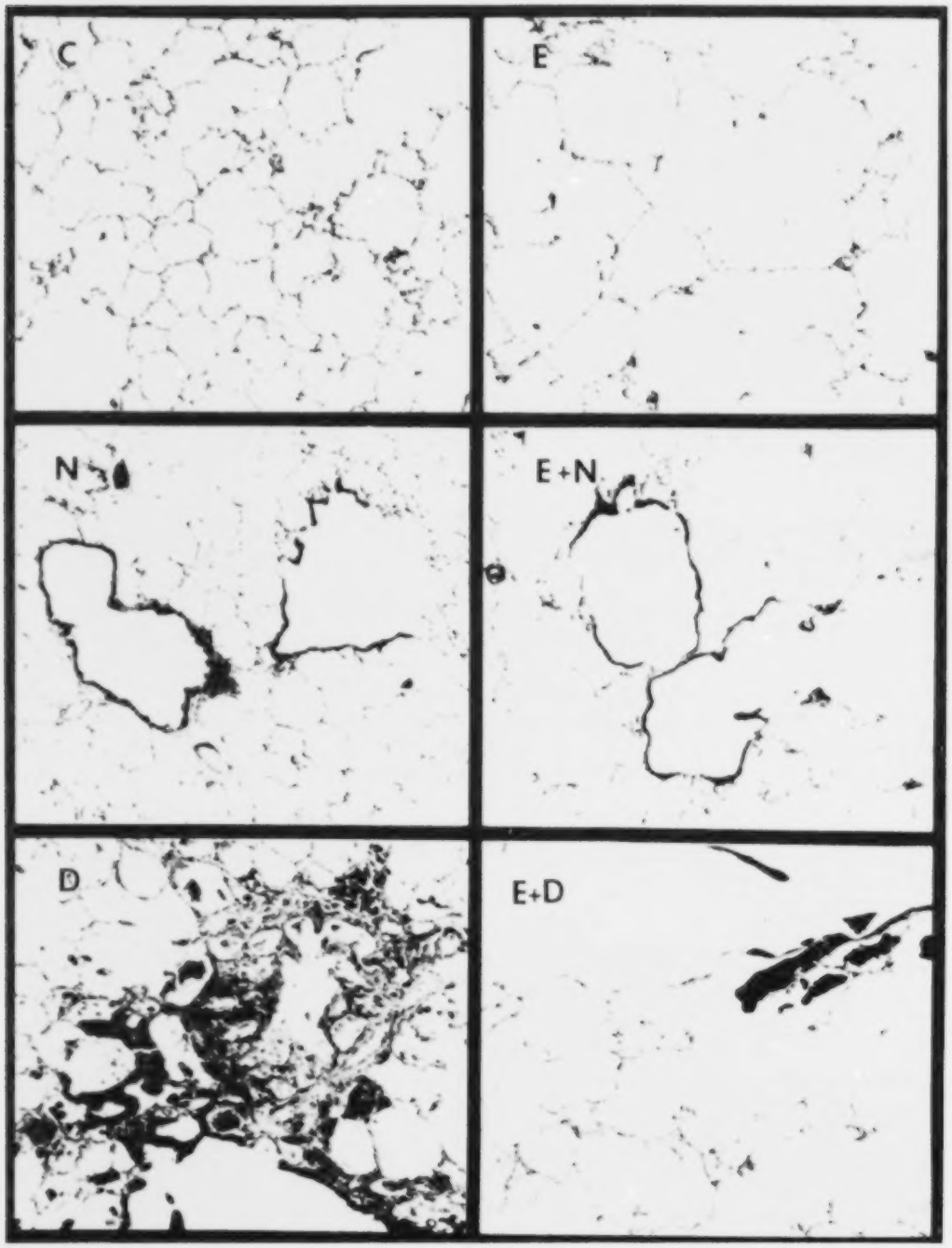

Figure 1. Photomicrographs of lung parenchyma of rats killed after 24 months of exposure or sham exposure (control). All lungs were fixed at 20 cm fixative pressure. Lungs of E and E + N rats appear similar to those of the corresponding C and N rats, except for enlargement of the airspaces. Lungs of E + D rats have similarly enlarged airspaces, but have less soot accumulation and less tissue response than lungs of D rats. Magnification ≈ × 99.

tion between emphysema and time was not significant. Other functional and morphological parameters were significantly influenced by both emphysema and time, but there was little evidence of progression. These results indicate that the disease was stable, progressing only slightly during the 24-month study.

The compositions of airway fluid from normal and emphysematous rats were not compared at base line. Subsequently, there was only one significant difference between values for the two groups, a greater level of acid proteinase activity after 12 months of exposure (Appendix E). Analysis of variance demonstrated significant influences of emphysema on several airway fluid parameters, for which concentrations were slightly higher in emphysematous control than in normal control rats at all measurement times, including total leukocytes, macrophages, lactate dehydrogenase, β-glucuronidase, glutathione reductase and peroxidase, acid proteinase, cathepsin B, and collagenous peptides. Although these slight differences persisted, they did not increase in magnitude with time. Total lung collagen was significantly greater in emphysematous than in normal rats at 24 months, but the amount of collagen per gram of lung weight was only slightly greater (Appendix F). An identical effect on total lung collagen was noted previously at this Institute (Harkema et al. 1984).

Emphysema had a small effect on the long-term clearance of radiolabeled particles inhaled at 18 months (Appendix H). The clearance half-time for E rats was 13 percent longer than that for C rats. The difference between these two groups was not significant by multiple comparison (Table H.1). Analysis of variance, however, demonstrated a significant influence of emphysema on clearance when all emphysematous groups were compared to all nonemphysematous groups (Table H.2). A prolonged clearance of particles having similar sizes was previously observed in emphysematous rats at this Institute (Lundgren et al. 1981).

EFFECT OF NITROGEN DIOXIDE ALONE

Three-way ANOVA indicated significant influences of NO_2 on several respiratory function parameters, including respiratory frequency, total lung capacity, vital capacity, functional residual capacity, residual volume, quasistatic chord compliance, CO diffusing capacity, forced vital capacity, the fraction of forced vital capacity exhaled in 0.1 second, and the volume-normalized values for peak expiratory flow, mean midexpiratory flow, and flow at 50 percent of forced vital capacity (Table D.4). The magnitudes of these changes were small. Multiple comparisons indicated no significant differences between values of N and C rats at 24 months (Table D.3). Lung volume was slightly increased, but the relative proportions of physiological subdivisions of lung volume remained normal. Although forced expiratory flow rates were normal, volume-normalized flow rates were slightly reduced because of the increased lung volume.

Three-way ANOVA indicated significant influences of NO_2 exposure on four airway fluid parameters: lactate dehydrogenase, alkaline phosphatase, glutathione reductase, and collagenous peptides all increased (Table E.4). Of these changes, multiple comparison indicated that only lactate dehydrogenase was significantly higher in N rats than in C rats at 24 months (Table E.3).

Two-way ANOVA indicated significant influences of NO_2 exposure on total lung collagen and collagen per gram of lung weight at 24 months (Table F.2). Although the values for N rats for both parameters was higher than those for C rats, only the change in total lung collagen was significant by multiple comparison (Table F.1).

Nitrogen dioxide exposure did not affect the clearance of radiolabeled particles (Appendix H).

Three-way ANOVA indicated significant influences of NO_2 exposure on lung weight and on lung weight normalized by body weight and lung volume (Table G.4). Although the values for N rats for these parameters were higher at 24 months than those for C rats, none of the differences was significant by multiple comparison (Table G.3).

The microscopic appearance of lungs from N rats was similar at all sacrifice times. At 12 months, there was mild hyperplasia of epithelium in terminal bronchioles, and an extension of bronchiolar epithelial cell types into proximal alveoli, giving the appearance of "respiratory bronchioles." Terminal bronchiolar walls were slightly thickened and eosinophilic. A slight inflammatory infiltrate of mixed cell type was occasionally found in alveoli adjacent to thickened bronchioles. Lesions at 18 and 24 months (see block N in Figure 1) were very similar to those observed at 12 months. The epithelialization of proximal alveoli appeared to progress slightly with time, but the inflammatory response remained minimal.

There was no evidence of progression of the effects of NO_2 on respiratory function or lung morphometry of N rats during the exposure. Analysis of variance indicated significant interactions between NO_2 and time for total protein, sialic acid, lactate dehydrogenase, and collagenous peptides in airway fluid. In summary, NO_2 exposure of rats with normal lungs caused mild cell injury and epithelial proliferation, mildly enlarged lungs, and an increased proportion of collagen in lung tissue.

EFFECT OF EMPHYSEMA ON RESPONSE TO NITROGEN DIOXIDE

The functional, biochemical, and structural effects of NO_2 exposure in emphysematous rats were qualitatively similar to those described above for normal rats. The microscopic appearance of emphysematous, NO_2-exposed lungs was similar to that of nonemphysematous NO_2-exposed lungs, except that alveoli and alveolar ducts were enlarged (see block E + N in Figure 1).

Significant interactions between emphysema and NO_2 exposure were indicated by ANOVA for only four parameters, two indices of forced expiratory flow during respiratory function tests and two proteolytic enzymes in airway fluid. The 24-month values of the C, E, N, and E + N groups for these parameters are listed in Table 5.

The flow difference at 25 percent of forced vital capacity is the difference between the actual flow rate at that lung volume and the flow representing a linear reduction of flow between 50 percent and 0 percent forced vital capacity. The parameter is an index of the shape of the flow-volume curve at low lung volumes, with negative values indicating a convex shape (normal) and positive values indicating a concave shape (characteristic of flow limitation). The mean values were negative for C and N rats, and positive for E and E + N rats. Only the difference between the values of the E + N and N rats was significant by multiple comparison. The expiratory flow rate at 10 percent forced vital capacity was slightly reduced by emphysema and further reduced by the combined treatment, but none of the differences was significant. Both acid proteinase and cathepsin B activities in airway fluid were increased above the control value in all treated groups, but none of the differences was significant at 24 months by multiple comparison.

The directions and magnitudes of the differences between mean values of the E, N, and E + N groups and those of the C group are presented in Table 6 for examination of the nature of the interactions between emphysema and NO_2 exposure. The flow difference at 25 percent forced vital capacity was increased in the E group and decreased in the N group, and was increased in the E + N group more than in the E group. The combined effect of emphysema and NO_2 was therefore more-than-additive. The expiratory flow at 10 percent forced vital capacity was decreased by both emphysema and NO_2, and the effect of the combined treatment was exactly additive. Acid proteinase was increased by both emphysema and NO_2; however, although the value of the E + N group was also increased, the effect was less-than-additive. Cathepsin B was increased by both treatments, and the combined effect was additive.

The above results indicate that the effects of the combined treatments were more-than-additive for only one of the four parameters for which ANOVA indicated a significant interaction between emphysema and NO_2 exposure. Of all the parameters measured, therefore, only the forced expiratory flow difference at 25 percent forced vital capacity met the criteria for indicating that emphysematous lungs might be more susceptible than normal lungs to the effects of NO_2 exposure. It is noteworthy that the difference between mean values of the E + N and E groups for this parameter was not significant at any measurement time (Tables D.1 through D.3); thus, the magnitude of this effect was similar in emphysematous rats regardless of NO_2 exposure.

Table 5. Effects, After 24 Months of Exposure, of Emphysema and Nitrogen Dioxide on Parameters for Which Analysis of Variance Demonstrated Significant Interactions Between Emphysema and Nitrogen Dioxide

		Control (C)		Emphysema (E)		Nitrogen Dioxide (N)		Emphysema + Nitrogen Dioxide (E + N)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Respiratory Function (n = 16)									
Flow difference at 25% forced vital capacity (FVC)	ml/sec	−3.2	7.1	1.5	4.7	−3.9	5.0	2.2[a]	4.1
Expiratory flow at 10% FVC (F_{10})	ml/sec	16	4	10	3	19	3	7	3
Airway Fluid (n = 8)									
Acid proteinase	activity/ml[b]	32	16	47	18	36	10	42	12
Cathepsin B	mIU/ml	0.022	0.001	0.032	0.019	0.027	0.013	0.037	0.020

[a] Difference from N mean significant at $p < 0.05$ by multiple comparison.

[b] Activity = micrograms of hemoglobin solubilized in four hours.

Table 6. Direction and Magnitude of Mean Differences from Control Mean Values, and Nature of Interaction, for Parameters Listed in Table 5

Parameter	Unit	Mean Difference from Control (C)			
		Emphysema (E)	Nitrogen Dioxide (N)	Emphysema + Nitrogen Dioxide (E + N)	Nature of Interaction[a]
Respiratory Function					
Flow difference at 25% FVC	ml/sec	+ 4.7	- 0.7	+ 5.4	+ +
F_{10}	ml/sec	- 6	- 3	- 9	+
Airway Fluid					
Acid proteinase	activity/ml	+ 15	+ 4	+ 10	-
Cathepsin B	mIU/ml	+ 0.010	+ 0.005	+ 0.015	+

[a] Interactions: + + = more-than-additive; + = additive; and - = less-than-additive. See Statistical Analyses in the Methods section for further definition.

EFFECT OF DIESEL EXHAUST ALONE

Exposure of rats with normal lungs to diesel exhaust caused adverse health effects of the type and magnitude expected, based on results of similar exposures in a previous study at this Institute (Bice et al. 1985; Henderson et al. 1985; Mauderly et al. 1987b, 1988; Wolff et al. 1987). The effects were not sufficiently severe to alter body weight or mortality. A progressive accumulation of soot in the lungs (Figure 2) was accompanied by a progressive focal fibrotic and proliferative lung disease (see block D of Figure 1). A mean of 12.1 mg of soot had accumulated in the lungs by 24 months of exposure. The lung burden of soot increased more steeply between 18 and 24 months of exposure than between 12 and 18 months. The increased rate of soot accumulation between 18 and 24 months was accompanied by a significant slowing of the long-term clearance of radiolabeled particles measured during the same period (18 to 22 months).

Exhaust exposure tended to decrease lung volumes, lung compliance, and CO diffusing capacity, but the relative proportions of the physiological subdivisions of volume were preserved. There was no evidence of airflow obstruction. Forced expiratory flow rates were either normal or increased, due to increased elastic recoil. Volume-normalized flows tended to increase because lung volumes were reduced.

Three-way ANOVA indicated significant influences of diesel exhaust on six respiratory function parameters, including reductions of quasistatic lung compliance and increases in mean midexpiratory flow and expiratory flow at 50 percent and 10 percent of forced vital capacity (Table D.5). The effects on expiratory flow rates were significant at 24 months by multiple comparison, but the effect on compliance was not (Table D.3).

Exhaust exposure altered airway fluid parameters progressively, as observed previously (Henderson et al. 1985). Increased neutrophils reflected an active inflammatory response, increased protein reflected vascular leakage, increased lactate dehydrogenase reflected cytotoxicity, increased alkaline phosphatase reflected type II cell injury, increased glutathione reductase and peroxidase reflected activation of mechanisms protective against foreign organic compounds and oxidants, increased acid proteinase and cathepsin B reflected proteolytic activity, and increased collagenous peptides reflected turnover of the extracellular collagen matrix.

Three-way ANOVA indicated that the influence of exhaust exposure was significant for all airway fluid parameters except total leukocytes and sialic acid, and that the influence of time was significant for all parameters (Table

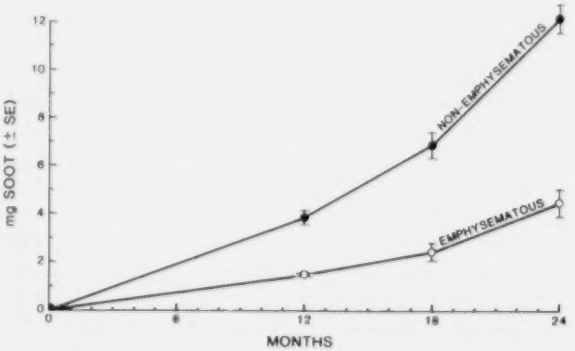

Figure 2. Amounts of diesel soot in lungs of nonemphysematous (D) and emphysematous (E + D) rats after 12, 18, and 24 months of exposure to diesel exhaust diluted to 3.5 mg soot/m^3. Values are plotted as means ± SE for five to eight rats per group. The lung burden of soot increased progressively in both groups; however, less soot was retained in lungs of E + D rats than in D rats. The complete data are presented in Appendix I.

E.5). All of the differences between values for D and C rats were significant by multiple comparison at 24 months (Table E.3).

Two-way ANOVA did not indicate a significant influence of exhaust exposure on lung collagen (Table F.2). Multiple comparison, however, indicated that the total lung collagen of the D rats was significantly higher than that of the C rats at 24 months, in approximate proportion to the increase in lung weight (Table F.1).

The half-time for clearance of radiolabeled particles from lungs of D rats between 18 and 24 months was twice that of C rats, and the difference was significant by multiple comparison (Table H.1). Two-way ANOVA indicated a significant influence of exhaust exposure on particle clearance (Table H.2).

After instillation of antigen at 24 months, the number of lymphocytes in pulmonary lymph nodes of D rats was significantly increased, but the proportion of cells producing antibody, the total number of antibody-forming cells, and the level of circulating antibody were slightly reduced (Table J.1). Similarly, two-way ANOVA indicated a significant influence of exhaust exposure on the number of lymphocytes, but not on the number or proportion of cells producing antibody (Table J.2).

Lung weight was slightly increased and lung volume was slightly reduced by exhaust exposure, resulting in a slightly increased lung density. The mean size of terminal airspaces was not altered; thus, internal surface area was slightly reduced. None of the effects of exhaust exposure on morphometric parameters was significant by multiple comparison at 24 months (Table G.3). Three-way ANOVA, however, indicated significant influences of exhaust exposure on lung weight and on lung weight normalized by body weight and lung volume (Table G.5).

The lesions observed microscopically progressed with time. At 12 months, individual macrophages containing soot were scattered throughout the lung. Focal aggregates of soot-laden macrophages were observed (fewer than 10 per section), primarily in alveoli adjacent to terminal bronchioles and beneath the pleura. Slight inflammatory cell infiltration and epithelial hypertrophy were associated with some foci of macrophages. The macrophage aggregates were larger and more numerous (15 to 20 per section) at 18 months, and epithelial hyperplasia was more pronounced. Alveolar septa near some foci were thickened with fibrous tissue and inflammatory cell infiltrate. Lesions were markedly more severe at 24 months (see block D of Figure 1). The frequency of macrophage aggregates was greater than 20 per section and the focal inflammatory response was more severe. Focal epithelial hyperplasia, squamous metaplasia, and fibrosis were prominent, and emphysematous changes were observed adjacent to some contracted fibrotic foci. A small bronchioloalveolar carcinoma was found in the lung of one rat. A progressive accumulation of soot-laden cells was also observed in the medullary and subcapsular sinuses of pulmonary lymph nodes.

The above observations indicate that the exhaust-induced lung disease progressed with exposure time. This progression was further demonstrated by the significant interactions between exposure and time obtained for several parameters by ANOVA.

EFFECT OF EMPHYSEMA ON RESPONSE TO DIESEL EXHAUST

The most striking difference between the responses of D and E + D rats to the exhaust exposure was the accumulation of less soot in the emphysematous lungs (Figure 2). The mean soot lung burdens of E + D rats were 39 percent, 36 percent, and 37 percent of the lung burdens of D rats at 12, 18, and 24 months, respectively. The focal lesions in the lungs of E + D rats were of the same type as those in D rats, but were less frequent and less severe (see block E + D in Figure 1). A patchy panacinar emphysema was evident, as in the other elastase-treated groups. Soot-laden macrophages were associated with a mixed inflammatory response, epithelial hyperplasia, and some fibrosis. The frequency of these foci varied widely within the group, ranging from approximately 5 to 15 per section. The lesions surrounding each focus were more severe in the rats with more numerous foci.

Most of the effects of exhaust exposure in E + D rats were qualitatively similar to the effects in D rats. The magnitudes of the effects, however, were generally less in E + D rats, in parallel with the accumulation of less soot in their lungs.

Significant interactions between the influences of emphysema and exhaust exposure were indicated by ANOVA for 19 parameters: 6 indices of respiratory function, 8 airway fluid parameters, total lung collagen, 3 morphometric parameters, and the half-time of radiolabeled particle clearance. There were no significant interactions for immunological parameters. The 19 parameters and the 24-month values for the C, E, D, and E + D groups are listed in Table 7.

Significant interactions between emphysema and exhaust exposure were found for the functional residual capacity: total lung capacity ratio, CO diffusing capacity and volume-normalized diffusing capacity, forced expiratory flow rate at 10 percent forced vital capacity and the volume-normalized flow, and the slope of the single-breath N_2 washout. Significant interactions were found for total protein, sialic acid, lactate dehydrogenase, β-glucuronidase, glutathione reductase and peroxidase, acid proteinase, and collagenous

Table 7. Effects, After 24 Months of Exposure, of Emphysema and Diesel Exhaust on Respiratory Function, Airway Fluid, and Lung Collagen Parameters for Which Analysis of Variance Demonstrated Significant Interactions Between Emphysema and Diesel Exhaust

		Control (C)		Emphysema (E)		Diesel Exhaust (D)		Emphysema + Diesel Exhaust (E + D)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Respiratory Function (n = 16)									
FRC/TLC	%	20	2	28[a]	4	22	4	28[a]	4
DL_{CO}	ml/min/mm Hg	0.265	0.041	0.250	0.078	0.254	0.047	0.269	0.057
DL_{CO}/lung volume	DL_{CO}/ml	0.016	0.003	0.012[a]	0.004	0.016	0.003	0.013[a]	0.003
F_{10}	ml/sec	16	4	10[a]	3	21[b]	3	10[a]	4
F_{10}/FVC	ml/sec/ml	1.0	0.4	0.6[a]	0.2	1.4[b]	0.2	0.6[a]	0.3
Slope of phase III of N_2 washout	% N_2/ml	0.43	0.07	0.47	0.10	0.49	0.11	0.45	0.07
Airway Fluid (n = 8)									
Total protein	mg/ml	0.14	0.04	0.18	0.08	0.37[b]	0.08	0.29	0.10
Sialic acid	nmol/ml	7.9	1.9	10.7	7.2	19.0[b]	4.9	10.5[a]	4.2
Lactate dehydrogenase	mIU/ml	74	28	102	58	311[b]	86	154[a]	40
β-Glucuronidase	mIU/ml	0.27	0.14	0.47	0.19	3.29[b]	0.71	1.23[a,b]	0.41
Glutathione reductase	mIU/ml	6.0	2.8	9.6	4.6	13.5[b]	3.8	9.3[a]	1.6
Glutathione peroxidase	mIU/ml	1.0	1.0	1.2	0.6	2.8[b]	1.1	2.5	1.5
Acid proteinase	activity/ml[c]	32	6	47	18	88[b]	13	67[a,b]	7
Collagenous peptides	µg/ml	4.1	0.6	3.9	1.3	10.4[b]	2.4	7.6[b]	3.1
Total Lung Collagen (n = 8)	mg	22.5	3.2	35.7[a]	7.1	32.4[b]	4.8	32.5	4.3
Morphometry (n = 8)									
Body weight	g	371	20	370	32	367	34	362	39
Lung weight	g	1.52	0.35	1.77	0.44	2.02	0.49	1.75	0.18
Lung weight/external lung volume	g/ml	0.10	0.03	0.09	0.02	0.15	0.05	0.09[a]	0.01
Clearance Half-Time of Radiolabeled Particles (n = 7–10)	days	52	8	59	8	109[b]	9	82[a,b]	13

[a] Difference from corresponding nonemphysematous group mean significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding non-diesel-exposed group mean significant at $p < 0.05$ by multiple comparison.

[c] Activity = micrograms of hemoglobin solubilized in four hours.

peptides in airway fluid. Significant interactions were found in the morphometric parameters of body weight, lung weight, and volume-normalized lung weight.

The direction and magnitude of the differences between the mean values of the E, D, and E + D groups and the mean values of the C group for parameters having significant emphysema-exhaust interactions are presented in Table 8. Among the 19 parameters, the interaction was more-than-additive for only 1, the body weight of rats killed for morphological evaluation. The mean body weights of the E, D, and E + D groups were all slightly reduced. The difference between the body weight of the E + D and C groups, however, was greater than the sum of the differences between the body weights of the E and C groups and the D and C groups.

Two points concerning the finding of more-than-additive influences of emphysema and exhaust exposure on body weight are noteworthy. First, the influences of all treatments on body weight were very small; none of the differences

Table 8. Direction and Magnitude of Mean Differences from Control Mean Values, and Nature of Interactions, of Parameters Listed in Table 7

Parameter	Unit	Mean Difference from Control (C)			
		Emphysema (E)	Diesel Exhaust (D)	Emphysema + Diesel Exhaust (E + D)	Nature of Interaction[a]
Respiratory Function (n = 16)					
FRC/TLC	%	+ 8	+ 2	+ 8	-
DL_{CO}	ml/min/mm Hg	- 0.015	- 0.011	+ 0.004	-
DL_{CO}/lung volume	DL_{CO}/ml	- 0.004	0	- 0.003	-
F_{10}	ml/sec	- 6	+ 5	- 6	-
F_{10}/FVC	ml/sec/ml	- 0.4	+ 0.4	- 0.4	-
Slope of phase III of N_2 washout	% N_2/ml	+ 0.04	+ 0.06	+ 0.02	-
Airway Fluid (n = 8)					
Total protein	mg/ml	+ 0.04	+ 0.23	+ 0.15	-
Sialic acid	nmol/ml	+ 2.8	+ 11.1	+ 2.6	-
Lactate dehydrogenase	mIU/ml	+ 28	+ 237	+ 80	-
β-Glucuronidase	mIU/ml	+ 0.20	+ 3.02	+ 0.96	-
Glutathione reductase	mIU/ml	+ 3.6	+ 7.5	+ 3.3	-
Glutathione peroxidase	mIU/ml	+ 0.2	+ 1.8	+ 1.5	-
Acid proteinase	activity/ml	+ 15	+ 56	+ 35	-
Collagenous peptides	µg/ml	- 0.2	+ 6.3	+ 3.5	-
Total Lung Collagen (n = 8)	mg	+ 13.2	+ 9.9	+ 10.0	-
Morphometry (n = 8)					
Body weight	g	- 1	- 4	- 9	+ +
Lung weight	g	+ 0.25	+ 0.50	+ 0.23	-
Lung weight/external lung volume	g/ml	- 0.01	+ 0.05	- 0.01	-
Clearance Half-Time of Radiolabeled Particles (n = 7–10)	days	+ 7	+ 57	+ 30	-

[a] Interactions: + + = more-than-additive, + = additive, - = less-than-additive. See Statistical Analyses in the Methods section for further definition.

among groups was significant at 24 months by multiple comparison (Table 7). Second, the significant interaction between body weight and exhaust exposure was found for only the small groups of rats killed for morphological study (n = 8 per group, per time). No similar interaction was found for body weight among the larger groups (n = 16 per group, per time) used for respiratory function evaluations.

The significant interactions between emphysema and exhaust exposure for all of the other 18 parameters were less-than-additive, regardless of the direction of change or whether emphysema or exhaust exerted the greater single influence (Table 8).

DISCUSSION

EFFECT OF NITROGEN DIOXIDE ALONE

The experimental design successfully achieved the desired distinct, but mild, effects of NO_2 exposure in rats with normal lungs. The nature and magnitude of the health effects produced by exposure to 9.5 ppm NO_2 for seven hours per day, five days per week were consistent with results from previous studies, as described below. The exposure, therefore, was considered suitable for testing the hypothesis that rats with emphysematous lungs have increased susceptibility to inhaled NO_2.

Survival was not affected in the present study. Freeman and coworkers (1972) found that continuous exposure (23 hours per day) of rats to 10 ppm NO_2 did not affect survival, but increased mortality was observed at 15 ppm. In a review of preceding studies of rats exposed to NO_2, Freeman and associates (1968a) noted that 10 ppm was the approximate upper limit for chronic exposures without increased mortality.

In the present study, airway fluid parameters reflected cell damage (lactate dehydrogenase and alkaline phosphatase) and stimulation of biochemical pathways that protect

against oxidants (glutathione) (Henderson et al. 1985; Henderson 1988). The same parameters were increased after rats were exposed for 15 weeks to NO_2 at 1 ppm, with twice-daily spikes to 5 ppm, in a previous study at this Institute (Gregory et al. 1983). In the present study, restructuring of the extracellular collagenous matrix was reflected by the increased collagenous peptides in airway fluid, and by the increased total lung collagen content (Pickrell et al. 1981). A dose-related alteration of lung collagen has been observed by other investigators. Stephens and coworkers (1971) found a subtle increase in the diameter of collagen fibrils in the terminal bronchiolar region of rats exposed for two years to 2 ppm NO_2, but did not measure collagen content. Lung collagen was significantly increased after rats were exposed for only two months to 20 ppm NO_2 for 24 hours per day, five days per week, in a previous study at this Institute (Pickrell et al. 1981).

The larger and heavier lungs of NO_2-exposed rats in the present study were consistent with previous observations. Haydon and associates (1965) found that exposing rats to 12 ppm NO_2 for 23 hours per day, seven days per week, for up to 32 months increased lung weight, but similar exposures at 4 ppm did not affect lung weight. Increased lung weight has been a consistent finding in all studies of rats exposed chronically to higher concentrations of NO_2. Few previous reports included data for lung volumes; however, both Freeman and coworkers (1972) and Juhos and coworkers (1980) reported increased in vivo lung volumes of rats exposed chronically to 15 to 16 ppm NO_2. Lung compliance was increased slightly in the present study, but not significantly, and only in proportion to the increase in lung volume. Freeman and colleagues also reported that although the lungs were larger, lung compliance was not significantly increased in rats exposed continuously to 2 ppm NO_2 for two years (Freeman et al. 1968b) or to 15 ppm for up to their life span (Freeman et al. 1972).

In the present study, focal epithelial hyperplasia occurred at the junction of the terminal bronchioles and alveolar ducts, resulting in a slight thickening of the terminal bronchiolar walls. Dose-related focal lesions in the region of the terminal bronchioles and alveolar ducts have been a consistent finding in rats exposed chronically to NO_2 at concentrations of 2 ppm (Freeman et al. 1968b) or higher (Freeman et al. 1972; Juhos et al. 1980). The epithelial lesions in the present study were evident by 12 months, and progressed little during the exposure period. The progression of the lesions may be dependent upon the concentration of NO_2 inhaled and the exposure pattern. Evans and associates (1972) reported that epithelial cell proliferation in the lungs of rats exposed continuously to 2 ppm NO_2 peaked during the first few days of exposure, and then remained near control rates for up to one year of exposure. The weekly concentration × time product of 322 ppm·hours per week achieved in that study was very close to the 333 ppm·hours per week achieved in the present study. In contrast, Juhos and coworkers (1980) reported that the lesions of rats exposed continuously to 15.7 ppm NO_2 (2,527 ppm·hours per week) progressed during 17 months of exposure.

The NO_2-induced "emphysema" reported to occur in some previous studies (Freeman et al. 1972) was not observed in the present study. In earlier reports (Haydon et al. 1965), "emphysema," defined as enlargement of alveoli without alveolar septal destruction, was reported to occur in rats exposed to NO_2 at concentrations below that used in the present study. The terminology applied to those findings is questionable. Emphysema is properly defined as an enlargement of terminal airspaces with destruction of alveolar septa (American Thoracic Society 1962). Later studies of rats exposed to higher concentrations of NO_2 (Freeman et al. 1972) produced alveolar enlargement with accompanying septal destruction, and loss of alveolar surface area. In the present study, alveolar diameter and surface area were not significantly affected by exposure (Appendix G).

Two factors might have contributed to this apparent difference in results. First, the exposures producing alveolar destruction were more severe than the exposure in the present study. The animals in the previous studies were exposed for 23 hours per day, seven days per week. At any NO_2 exposure concentration higher than 2 ppm, this exposure pattern would have produced a weekly concentration × time product higher than that of the present study. The utility of comparing exposures to reactive gases solely on the basis of concentration × time products is questionable. Perhaps more importantly, the exposures in the previous studies were nearly continuous, while those in the present study were only seven hours per day, five days per week. Second, none of the previous studies documented the microbiological status of the animals. Lung infections with various microorganisms are thought to have been common in rodents in past inhalation toxicological studies, and their potential contribution to the pathogenesis of lesions ascribed to the exposure is unknown.

EFFECT OF EMPHYSEMA ON RESPONSE TO NITROGEN DIOXIDE

Only 1 of a total of 61 measured parameters gave results meeting the criteria for suggesting that emphysematous rats might have increased susceptibility to NO_2 exposure. The other parameters demonstrated either additive influences of the two treatments (2 parameters), less-than-additive influences (1 parameter), or no significant interaction be-

tween the two treatments (57 parameters). Furthermore, the relationship suggested by the single parameter, the forced expiratory flow difference at 25 percent forced vital capacity, was not supported by the several other indices of forced flow limitation obtained during the same measurement. On the basis of these findings, the results are not consistent with the hypothesis that rats with preexisting pulmonary emphysema are more susceptible to the adverse effects of chronic NO_2 inhalation than rats with normal lungs.

The finding that the effects of chronic inhalation of NO_2 are similar in nature and magnitude in emphysematous and normal lungs is consistent with the previous finding at this Institute that emphysematous and normal rats have similar susceptibilities to acute oxygen toxicity (Harkema et al. 1982). The emphysematous lung appears to respond to inhaled oxidant gases in a manner similar to that of the normal lung. These findings are also consistent with the conclusion, reached in the review by Snider and coworkers (1986), that emphysematous lungs are no more susceptible than normal lungs to injury from gaseous and water-soluble irritants.

The fact that emphysematous and normal lungs appear to be at equal risk from NO_2 inhalation implies that individuals with emphysema might still constitute a group of special concern with regard to inhalation exposures. Emphysema and NO_2 exposure altered several functional, biochemical, and morphometric parameters in the same direction; for example, both treatments resulted in larger and heavier lungs. In general, rats receiving both treatments were more adversely affected than rats receiving either single treatment. In this sense, the emphysematous rats were at greater risk from NO_2 exposure than normal rats because their health was already compromised. The NO_2 exposure acted to accentuate the health impairment of the emphysematous rats. Therefore, although it was shown that emphysematous rats were not more susceptible to the effects of NO_2 exposure than normal rats, the results suggest that individuals with preexisting emphysema might be at greater risk from chronic, high-level NO_2 exposures than individuals with otherwise normal lungs.

EFFECT OF DIESEL EXHAUST ALONE

The experimental design successfully achieved the desired distinct, but mild, effects of exhaust exposure in rats with normal lungs. The effects observed in nonemphysematous exhaust-exposed rats were similar to those observed in identically exposed rats in a previous study at this Institute, and to those observed by other investigators. The exposure, therefore, was considered suitable for testing the hypothesis that rats with emphysematous lungs have increased susceptibility to inhaled diesel exhaust.

The amount of soot in the lungs at the end of the present 24-month exposure, 12.1 mg, was similar to the 11.5-mg lung burden of rats previously exposed to 3.5 mg soot/m^3 for 24 months at this Institute (Mauderly et al. 1987b; Wolff et al. 1987). Researchers at General Motors measured lung burdens of soot in male Fischer-344 rats exposed 20 hours per day, 5.5 days per week, for up to 18 months, to exhaust from 5.7-liter Oldsmobile diesel engines operated in a steady-state mode (Strom 1984; Strom et al. 1985). The lung burdens observed by General Motors might best be compared to those observed in the present study on the basis of micrograms of soot accumulated in the lung per milligram hour per cubic meter of exposure, because both the exposure concentration and the pattern differed between the studies. The lungs of rats exposed for 18 months at a concentration of 3.5 mg soot/m^3 in the present study had accumulated 0.72 µg soot/mg·hr·m^{-3} soot exposure, while rats exposed by General Motors for 18 months at a concentration of 1.5 mg soot/m^3 had accumulated approximately 0.87 µg soot/mg·hr·m^{-3} (Strom et al. 1985). This comparison suggests that the exposure pattern, as well as the concentration, influences the accumulation of diesel soot in the lung. The approximately 20 percent greater accumulation of soot relative to exposure in the rats in the General Motors study probably resulted from their being exposed for 110 hours per week (65 percent of total time), in contrast to the 35 hours per week (21 percent of total time) that the rats in the present study were exposed.

An increase in the rate of accumulation of soot during the last six months of exposure and a concurrent slowing of clearance of radiolabeled particles were also observed in a previous study at this Institute (Wolff et al. 1987). An impairment of clearance of labeled particles from the lungs of rats chronically exposed to diesel exhaust has also been demonstrated by others. Chan and coworkers (1984) found the clearance of radiolabeled diesel particles to be slowed after rats were exposed for 20 hours per day, for up to 102 days, to exhaust at a concentration of 6 mg soot/m^3. Heinrich and associates (1986) exposed Wistar rats to whole diesel exhaust at 4.2 mg soot/m^3 for 19 hours per day, five days per week, for their life span, and found the long-term clearance half-time of inhaled ^{59}Fe-labeled iron oxide particles to be increased by approximately 50 percent in the exposed rats.

The histological appearance of the lungs of exhaust-exposed rats was consistent with that observed in all studies of rats exposed chronically to high levels of diesel exhaust (White and Garg 1981; Pepelko and Peirano 1983; Brightwell et al. 1986; Heinrich et al. 1986; Ishinishi et al. 1986;

Lewis et al. 1986; Mauderly et al. 1988). Chronic exposure of rats to high levels of exhaust consistently results in a focal, chronic, active inflammatory response centered around accumulations of soot-laden macrophages. The fibrotic and proliferative lesions, which result in progressively heavier lungs, are dose- and time-related. Exposure for 24 months or longer to high concentrations of exhaust also results in an increased incidence of lung tumors of epithelial origin in rats observed for their life span (Brightwell et al. 1986; Heinrich et al. 1986; Ishinishi et al. 1986; Mauderly et al. 1987b). The size of the treatment groups in the present study was not sufficient to evaluate carcinogenicity; thus, the relation between exhaust exposure and the single tumor observed in the D group is unknown.

The reduced physiological lung volumes, slightly reduced lung compliance, and lack of forced airflow limitation observed in the present study were consistent with the functional changes observed previously at this Institute (Mauderly et al. 1988). The thickening of alveolar walls by cell proliferation and fibrosis resulted in a stiffer lung (reduced compliance); thus, the volume of the lung inflated to a standard pressure was reduced. The increased elastic recoil placed greater radial tension on small airways and increased the driving pressure at the beginning of forced exhalation. These influences, and the lack of pathology in conducting airways, would have acted to maintain normal forced airflow, with increased flow relative to the reduced lung volume.

Gross (1981) exposed Fischer-344 rats for 20 hours per day, 5.5 days per week, to diesel exhaust diluted to 1.5 mg soot/m^3. After 612 days of exposure, he observed nonsignificant, but consistent, trends toward reduced dynamic lung compliance and smaller lung volumes, but no restriction of forced airflow. The weekly soot concentration × time product in that study was similar to that in the present study, and the changes in respiratory function were similar to those at a similar time in the present study (18 months). Heinrich and coworkers (1986) observed a significant reduction of dynamic lung compliance of rats after 24 months of exhaust exposure, while compliance was only slightly reduced in the present study. The greater effect in the Heinrich study was probably related to the more severe exhaust exposure, which achieved a weekly soot concentration × time product for soot exposure 3.2 times that of the present study.

Changes in the airway fluid parameters in the present study reflected the exposure-related cytotoxicity and collagen turnover observed previously at this Institute (Henderson et al. 1985). Heinrich and colleagues (1986) reported similar changes in rats exposed for 24 months to diesel exhaust. The magnitudes of the increases in the concentrations of five parameters common to both studies (lactate dehydrogenase, alkaline phosphatase, total protein, acid proteinase, and collagen) were greater in the Heinrich study, in parallel to the greater concentration × time product of that study.

In the present study, the significant increase in cellularity of the pulmonary lymph nodes, without significant change in cellular antibody formation or in circulating IgM antibody level, was consistent with previous findings at this Institute (Bice et al. 1985). The increase in lymphoid cell number was qualitatively associated with the accumulation of soot in the nodes. Dziedzic (1981) reported that the numbers of T- and B-lymphocytes were not increased in the pulmonary lymph nodes of guinea pigs exposed to diesel exhaust 20 hours per day, 5.5 days per week, for up to eight weeks at a soot concentration of 1.5 mg/m^3, but did not make observations after longer exposures. No other studies have examined pulmonary immune responses after prolonged exposure to diesel exhaust.

EFFECT OF EMPHYSEMA ON RESPONSE TO DIESEL EXHAUST

Only 1 of a total of 65 measured parameters gave results meeting the criteria for suggesting that rats with emphysematous lungs might be more susceptible than rats with normal lungs to the effects of diesel exhaust exposure. The other parameters all demonstrated less-than-additive influences (18 parameters), or no significant interaction between the two treatments (46 parameters). Furthermore, the relationship suggested by the single parameter, the body weight of rats killed for morphology, was not supported by the data for body weight of larger numbers of rats used for respiratory function tests. On the basis of these findings, the results are not consistent with the hypothesis that rats with preexisting pulmonary emphysema are more susceptible than rats with normal lungs to the adverse effects of chronic diesel-exhaust inhalation.

Rats with pulmonary emphysema were generally affected less by exposure to diesel exhaust than rats with normal lungs, in parallel with the accumulation of less diesel soot in the lungs of emphysematous rats. Although the lungs of emphysematous rats were adversely affected by the exhaust exposure, emphysema acted to spare the rats from the full effects of exhaust.

The only other reported study of normal and emphysematous rats exposed chronically by inhalation to relatively insoluble particles demonstrated a similar association between reduced particle accumulation and reduced lung injury in emphysematous rats. Gross and deTreville (1969) induced emphysema in rats by intratracheal instillation of

papain four months before beginning inhalation exposures to quartz at 310 mg/m^3 or bituminous coal dust at 770 mg/m^3, six hours per day, five days per week, for 115 exposure days over a one-year period (alternate months with and without exposure). Rats without emphysema were similarly exposed. The emphysema was very nonuniform, and the authors noted that both the amount of particles and the severity of disease were greater in regions without airspace dilation. They later reported (Gross et al. 1971) that the amounts of quartz and coal dust in the lungs of the emphysematous rats were 76 and 80 percent, respectively, of the amounts in the lungs of nonemphysematous exposed rats at the end of exposure. In parallel, the lung weight and lung contents of hydroxyproline (collagen) and lipid in emphysematous quartz-exposed rats were 77, 90, and 63 percent, respectively, of the values for nonemphysematous quartz-exposed rats. In contrast, the increase in lung weight was greater in emphysematous than in nonemphysematous coal-exposed rats. No data were given for the effect of coal exposure on other parameters. Hamsters were also included in the study, and lung burdens of quartz and coal dust in emphysematous hamsters were only 41 and 51 percent, respectively, of the lung burdens of nonemphysematous hamsters.

A few shorter-term studies of emphysematous and normal rats exposed by inhalation to particles have been reported. The EPA reported two studies in which animals were exposed for one month. Raub and coworkers (1983) exposed hamsters 23 hours per day for 28 days, beginning four weeks after instillation of elastase, to a reaction mixture of olefin, ozone, and sulfur dioxide. The resulting 0.3-μm particles were primarily ammonium sulfate. The study yielded uncertain information on the effects of emphysema. Epithelial hyperplasia in the terminal bronchiolar region was greater in emphysematous hamsters than in nonemphysematous hamsters. The CO diffusing capacity was increased in nonemphysematous hamsters, but not in emphysematous hamsters, and the authors concluded that emphysema prevented the hamsters from responding to the injury by increasing gas exchange. Busch and coworkers (1984) exposed rats and guinea pigs six hours per day, five days per week, for four weeks to ammonium sulfate particles at 1 mg/m^3, beginning three weeks after instillation of elastase. Both emphysema and exposure increased the size of alveoli. A significant antagonistic interaction between the treatments was demonstrated for alveolar size in guinea pigs. Researchers at the University of Kentucky examined interactions between elastase-induced emphysema and cigarette smoke inhalation. The focus was on the influence of smoke on the evolution of the emphysema. Kimmel and associates (1985) and Lai and Diamond (1986) exposed rats to cigarette smoke for 12 weeks, beginning three days after instillation of elastase, and reported that elastase and smoke interacted to increase the severity of emphysema.

The results of the present study, and those of Gross and associates (1971), suggest that the primary reason for the reduced susceptibility of emphysematous animals to chronically inhaled toxic dusts was the reduced accumulation of particles in the lungs of emphysematous subjects. The accumulation of dust lung burdens is determined by the exposure concentration × time product and the competing actions of deposition and clearance (see Lippmann et al. 1980 for a review of deposition and clearance phenomena). Since the exposures of the normal and emphysematous rats were identical (housed in the same chamber), deposition, or clearance, or both, must have differed between the groups. The lung burden of soot measured in the present study included material in intrapulmonary airways, the interstitium, and lymphatic channels. The pulmonary lymph nodes were removed before measurement. Total pulmonary deposition is determined by the total volume of inhaled aerosol reaching the pulmonary region and the fraction that deposits in the pulmonary region. Although respiration of the conscious rats was not measured in the present study, data collected during respiratory function tests did not suggest that the minute volumes of the normal and emphysematous rats differed significantly. The fractional deposition, therefore, probably differed between the groups. Clearance of small particles from the lung primarily involves mucociliary activity in ciliated airways, phagocytosis by alveolar macrophages and movement to the airways or interstitium, and dissolution of the particles. Macrophage-mediated clearance is thought to have been the primary determinant of clearance in diesel-exhaust-exposed rats, since diesel soot has low solubility in biological fluids (Brooks et al. 1981). In addition, tracheal mucociliary clearance has been shown to be affected little by chronic diesel-exhaust exposure (Wolff et al. 1987), and the particles are seldom observed outside of phagocytes in the lung or lymph nodes.

Movement of particles to airspace walls by diffusion and sedimentation is the predominant mechanism for deposition of fine particles in alveoli (Yu and Taulbee 1977; Lippmann et al. 1980). We hypothesize that the greater distance for diffusion and sedimentation in the emphysematous lungs, due to enlarged alveoli and alveolar ducts, was the primary cause of the lesser accumulation of soot in the lungs of E + D rats than in the lungs of D rats. Deposition of soot particles was not measured in the present study. The initial lung burdens of the ^{134}Cs-labeled fused aluminosilicate particles inhaled at 18 months, however, provide some insight into the relative deposition efficiencies of the E + D

and D rats. The mean initial lung burden of radiolabeled particles of all emphysematous groups combined was 72 percent of the lung burdens of all normal groups combined. Diffusion would be expected to exert a greater influence on deposition of the 0.25-μm soot particles than on the 1.0-μm aluminosilicate particles. The difference in deposition between D and E + D rats, therefore, was probably greater for soot than for the radiolabeled particles.

There are other reports of reduced lung burdens of inhaled particles in emphysematous rats, although none includes data for deposition of particles as small as diesel soot. Gross and coworkers (1971) (study described above) reported that the amounts of 1.4-μm coal and 2.4-μm quartz particles in lungs of chronically exposed emphysematous rats were 80 and 76 percent, respectively, of the amounts in lungs of normal rats. Ferin (1971) reported that the initial lung burden of 1.5-μm titanium dioxide particles in emphysematous rats after a single exposure was 50 percent of the lung burden in normal rats. Lundgren and colleagues (1981) reported that the initial lung burden of emphysematous rats exposed once to 2.2-μm $^{239}PuO_2$ particles was 63 percent of the lung burden of normal rats. Damon and associates (1983) reported that the initial lung burden of emphysematous rats exposed once to 1.2-μm ^{59}Fe-labeled iron oxide particles was 45 percent of the lung burden of normal rats. The finding of the greatest difference in deposition between emphysematous and normal rats in the Damon study, which used the smallest particles, is consistent with the idea that diffusion path length has an important influence.

Reduced pulmonary deposition of particles in emphysematous lungs has also been observed in hamsters. Gross and colleagues (1971) reported that lung burdens of coal and quartz in chronically exposed emphysematous hamsters were 51 percent and 41 percent, respectively, of the lung burdens of nonemphysematous hamsters. Hahn and Hobbs (1979) reported that the initial lung burden of hamsters with elastase-induced emphysema that were exposed once to 1.5-μm ^{137}Cs-labeled fused aluminosilicate particles was 45 percent of the lung burdens of normal hamsters. Sweeny and coworkers (1987) reported that the amount of 0.46-μm radiolabeled sulfur colloid particles deposited in the lungs of hamsters with elastase-induced emphysema was 85 percent, 84 percent, and 71 percent of the amount deposited in the lungs of normal hamsters at 30, 60, and 90 days, respectively, after induction of emphysema. This latter study examined the effect of emphysema on deposition of the smallest particles used to date, particles that were approximately twice the size of the soot particles used in the present study (0.25 μm).

The difference in the rate of particle clearance between D and E + D rats would also have contributed to their differences in soot lung burden. Long-term clearance was 25 percent faster in E + D rats than in D rats after 18 months of exposure. It could be argued either that faster clearance caused a smaller lung burden, or that the smaller lung burden allowed a faster clearance. Regardless, the faster clearance in E + D rats during the latter months of exposure is consistent with both their lower lung burden and their less steep increase in lung burden between 18 and 24 months (Figure 2).

The half-time of the long-term clearance component of radiolabeled particles was 17 percent greater in emphysematous control (E) rats than in normal control (C) rats (Appendix H). This increase reflected the influence of emphysema alone. The clearance half-time of the E + D rats was 39 percent longer than that of the emphysematous control (E) rats, reflecting the influence of diesel exhaust. The clearance half-time of the E + D rats was 58 percent longer than that of the normal control (C) rats, an increase almost identical to the sum of the separate increases due to emphysema and diesel exhaust (56 percent). The two effects, therefore, were additive.

Other investigators have reported that emphysema slows the long-term clearance of inhaled particles in animals. Ferin (1971) reported a 67 percent greater retention of titanium dioxide particles in emphysematous than in normal rats at 25 days after a single exposure. Hahn and Hobbs (1979) reported a 31 percent longer half-time for the third component of a three-component fit to whole-body clearance of radiolabeled particles from the lungs of elastase-treated hamsters than from normal hamsters after a single exposure. Lundgren and colleagues (1981) reported that the half-time of the second component of a two-component fit to whole-body retention of $^{239}PuO_2$ was 30 percent longer in emphysematous than in normal rats after a single exposure. Damon and coworkers (1983) reported a 10 percent longer half-time for clearance of iron oxide particles from the lungs of emphysematous than from normal rats after a single exposure.

The results of the present study do not suggest that individuals with pulmonary emphysema might be more susceptible than individuals with normal lungs to the effects of exposure to high concentrations of diesel exhaust. The effects of exhaust exposure were expressed in emphysematous rats, but to a lesser degree than in rats with nonemphysematous lungs. This effect is attributed to the reduced deposition of diesel soot in the lungs of the emphysematous rats. The relevance of this finding for estimates of the effects of diesel exhaust in emphysematous humans would appear to depend on whether or not soot deposition would be less in emphysematous than in normal humans. As noted above, a deficiency of the elastase-induced model of emphysema

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is its failure to mimic the full range of structural and functional abnormalities that are present in the most common forms of human pulmonary emphysema. Little is known about the deposition and retention of 0.25-μm particles in the lungs of humans with different types of emphysema. A fuller understanding of the implications of the present study for human health effects awaits the collection of such data.

The finding that emphysematous rats did not have increased susceptibility to diesel exhaust is consistent with the earlier finding at this Institute that emphysematous rats were not more susceptible than normal rats to acute oxygen toxicity (Harkema et al. 1982), and with the reports by Raub and coworkers (1983) and Busch and coworkers (1984) that animals with emphysema were not more susceptible than normal animals to short-term exposures to irritant particles. In these studies, as in the present study, the exposures caused cytotoxicity and inflammation. The emphysematous lung is apparently not at increased risk from these influences.

Although emphysematous lungs were affected less by exhaust than were normal lungs, the results of this study suggest that individuals with emphysema might constitute a group of special concern with regard to exhaust inhalation. The emphysematous lungs were damaged by chronic exposure to high concentrations of diesel exhaust, and the condition of the exposed emphysematous lungs was worse than that of the unexposed emphysematous lungs. Any additional injury, regardless of source, would further compromise the health of subjects with pulmonary emphysema. Since emphysematous lungs have less reserve functional capacity than do normal lungs, the effects of exposure (of identical magnitudes) might be more threatening to emphysematous than to normal subjects. These findings suggest that individuals with pulmonary emphysema should avoid prolonged exposures to high concentrations of diesel exhaust.

SUMMARY

The results of this study suggest that rats with preexisting pulmonary emphysema were not more susceptible to the effects of chronic inhalation exposure to NO_2 or diesel exhaust than were rats with normal lungs. Mild, but distinct, adverse health effects were induced in normal and emphysematous rats alike by both exposures. These effects were consistent with those observed in previous studies. Among the many health effects parameters measured, significant interactions between emphysema and exposure were demonstrated by analysis of variance for only 4 parameters for NO_2 exposure and 19 parameters for exhaust exposure. For each exposure, only one of these interactions was more-than-additive, and neither was supported by changes in related parameters.

Several of the effects of emphysema and NO_2 exposure were similar and tended to be additive. These included effects on respiratory function and airway fluid indicators of injury. The health of emphysematous NO_2-exposed rats, therefore, was compromised to a greater degree than the health of rats receiving either treatment alone.

The health of emphysematous exhaust-exposed rats was also compromised more than that of rats with emphysema alone, but less than that of nonemphysematous rats exposed to exhaust alone. This was thought to have been primarily due to the accumulation of less diesel soot in the lungs of emphysematous rats. The reduced accumulation of soot most probably resulted from reduced pulmonary deposition by diffusion and sedimentation, due to enlarged alveoli. In addition, some of the effects of emphysema and exhaust acted in opposite directions, and thus tended to be counteractive.

The adverse effects of NO_2 and exhaust exposure resulted in more severely compromised health in exposed rats with preexisting pulmonary emphysema than in nonexposed emphysematous rats.

The finding that emphysematous rats responded similarly to nonemphysematous rats when exposed to NO_2 or exhaust was consistent with the results of previous studies of emphysematous animals exposed by inhalation to oxidant gases and irritant particles.

SIGNIFICANCE OF FINDINGS

The findings of this study suggest that the health effects of chronic inhalation of NO_2 in humans with pulmonary emphysema would be qualitatively and quantitatively similar to those in humans with normal lungs. The findings suggest that the health effects of inhalation of diesel exhaust would be qualitatively similar, but might be quantitatively less in emphysematous than in normal subjects. The findings suggest that the effects in humans of pulmonary emphysema and chronic inhalation exposure to NO_2 or diesel exhaust might not be more-than-additive.

It is important to understand that these findings do not suggest that humans with emphysema do not constitute a group of special concern with respect to risk from inhaled pollutants. The respiratory health of the emphysematous exposed rats was more severely compromised than that of the unexposed emphysematous rats. In the case of NO_2, the health of the emphysematous exposed rats was also more se-

verely compromised than that of the nonemphysematous exposed rats. Although the effects of emphysema and exposure may not be more-than-additive (the hypothesis tested by this study), chronic exposures to high levels of the pollutants would be expected to result in adverse effects that would be superimposed on the preexisting effects of emphysema. Thus, individuals with pulmonary emphysema (and perhaps other lung diseases) would clearly constitute a group of special concern.

The finding that the combined effects of emphysema and pollutant exposure are either worse (NO_2), or not worse (diesel exhaust), than the effects of exposure alone has an important bearing on the qualitative aspects of risk assessment. The finding that the effects of emphysema and exposure are not more-than-additive has an important bearing on the quantitative aspects of risk assessment.

The nature of the experimental model of pulmonary emphysema used in this study is a major consideration when extrapolating the findings of this study to humans. The pulmonary lesions of the elastase-treated rats clearly met the definition of emphysema: enlarged terminal airspaces with destruction of alveolar septa. The lesions were stable throughout the study period, however, and were not accompanied by inflammation or pathology of conducting airways. Emphysema in humans is seldom stable, but is typically progressive and involves a continuous restructuring of the lung parenchyma. In addition, emphysema in humans occurs most frequently in cigarette smokers, and is accompanied by chronic bronchitis. The dynamic, evolving emphysematous lesions of humans might interact with the effects of chronic inhalation exposures to NO_2 or diesel exhaust in ways that the more quiescent lesions of the rats in the present study did not. This experimental limitation was recognized before the present study was initiated. In the absence of a more representative model of human emphysema, however, it was felt that the present study would contribute to our understanding of potential interactions between emphysema and the exposures.

The results of this study point toward the need for information on the deposition of inhaled particles in abnormal lungs, and the deposition of fine particles in emphysematous lungs in particular. Although this issue is recognized among researchers in the field and some data have been obtained, substantial gaps in our knowledge remain.

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APPENDIX A. Rationale for Selecting Health Effects Evaluations

The assays used to evaluate health effects were selected on the basis of their demonstrated usefulness in previous studies of inhaled materials and lung injury at this Institute and elsewhere, and for their relevance to parameters measured in humans.

Respiratory function is the primary endpoint used in studies of the effects of airborne toxicants on humans, in both epidemiological and experimental exposure settings. Most assays of respiratory function applied to humans have been adapted to laboratory animals. Methodological and interpretation issues have been reviewed in detail (Costa 1985; Mauderly 1989). Good correspondence has been demonstrated between humans and animals in the relations between altered lung structure and function (Mauderly 1984a; Mauderly 1988). The respiratory function tests used in the present study have been shown at this Institute to be sensitive to changes in rats associated with aging (Mauderly et al. 1982, 1984a), elastase-induced pulmonary emphysema (Harkema et al. 1982), bleomycin-induced pulmonary fibrosis (Mauderly 1988), oxygen toxicity (Harkema et al. 1982), starvation (Harkema et al. 1984), histamine-induced airway constriction (Likens and Mauderly 1982), quartz inhalation (Benson et al. 1985), diesel-exhaust inhalation (Mauderly et al. 1988), and powdered-dye inhalation (Sun et al. 1987).

The analysis of airway fluid was considered important for the information it yields on lung injury and the lung's response to injury. The parameters measured and interpretation of results have been discussed in detail (Henderson et al. 1981, 1985; Henderson 1984), and the role of airway fluid analysis in toxicology has been recently reviewed (Henderson 1988). Total and differential cell counts reveal inflammatory responses. Release of cytoplasmic enzymes reflects cytotoxicity. Increases in lysosomal enzymes reflect active phagocytosis. Increased total protein usually reflects leakage from pulmonary vasculature. Increases in other enzymes reflect protective responses against inhaled oxidant and organic compounds. The level of cathepsin B reflects tissue acid proteolytic activity, involved in the remodeling of lung structure. These parameters have been shown at this Institute to be useful indicators of responses of rodents to injury from numerous inhaled toxicants, including NO_2 (DeNicola et al. 1981) and diesel exhaust (Henderson et al. 1985).

The evaluation of lung collagen turnover was considered important as an indicator of the remodeling of lung structure at the biochemical level. The concentration of hydroxyproline-containing collagenous peptides in airway fluid was measured each time rats were killed, as a serial indicator of the rate of turnover of the extracellular collagenous matrix. The assay for airway collagenous peptides is much less time-consuming and expensive than the assay for lung tissue collagen, and the level of airway collagenous peptides was previously shown at this Institute to be a good indicator of increasing lung collagen in rats with oxidant-induced lung injury (Pickrell et al. 1987). The actual amount of collagen in lung tissue was measured after 24 months of exposure to quantify the extent of collagen accumulation as a result of exposure. Lung collagen was previously shown at this Institute to result from repeated exposure of rats to NO_2 (Pickrell et al. 1981) and diesel exhaust (McClellan et al. 1986), and to be increased in rats with elastase-induced pulmonary emphysema (Harkema et al. 1984). The cellularity of pulmonary lymph nodes and their immune responses to instilled particulate antigen were previously shown at this Institute to be altered by chronic inhalation exposure of rats to diesel exhaust (Bice et al. 1985). The effects occurred in parallel with the accumulation of soot particles in the nodes, and were thought to be related to the amount of soot translocated to the nodes. These assays were performed on control and exhaust-exposed rats, after 24 months of exposure, as functional reflections of potential emphysema-related differences in the translocation of soot to the lymph nodes.

The rate of clearance of radiolabeled particles from the lungs of all groups was measured between 18 and 24 months of NO_2 or exhaust exposure. The usefulness of this assay was based on the previous finding at this Institute (Wolff et al. 1987) that chronic exposure to diesel exhaust slowed the clearance of tracer particles. Although acute exposures to oxidant gases and irritant materials are known to affect particle clearance, it was not known if chronic exposure to NO_2 would affect clearance. Previous work at this Institute (Lundgren et al. 1981) demonstrated that rats with elastase-induced emphysema cleared inhaled particles more slowly than rats with normal lungs; thus, the interactive effects of emphysema and toxicant exposure on clearance were of interest.

The methods used to quantify morphological changes in the lungs were based on previous experience that chronic exposure to diesel exhaust (McClellan et al. 1986; Mauderly et al. 1988) and NO_2 (Pickrell et al. 1981) altered lung weights, volumes, and terminal airspace diameters. It was also known from previous work (Harkema et al. 1982, 1984; Likens and Mauderly 1982) that elastase-induced emphysema altered these parameters. The interactions between emphysema and toxicant exposure were of primary interest. In addition to the rationale presented above, the health ef-

fects assays used in the present study were selected for correspondence with those used in a companion HEI-sponsored study of the effects of age on susceptibility to NO_2 and diesel exhaust (Mauderly et al. 1987b).

APPENDIX B. Methods for Generating and Characterizing Exposure Atmospheres

NITROGEN DIOXIDE GENERATION

Gaseous NO_2 was generated by controlled evaporation of liquid nitrogen tetroxide (N_2O_4) dimer and subsequent dilution with clean air. An evaporation flask was seated in a water bath containing an ethylene glycol and water mixture and cooled below the freezing point of water. A stopper in the top of the flask contained a tube for admitting a stream of pure, dry N_2 from a cylinder via a mass flow controller. Another tube in the stopper carried the vaporized NO_2 gas in the N_2 stream to an injection point in the pipe carrying filtered air to the inlet of the chamber. The rate of NO_2 generation was controlled by the flow of carrier N_2 and the temperature of the cooling bath.

MEASUREMENT OF NITROGEN DIOXIDE IN THE EXPOSURE CHAMBER

A chemiluminescent analyzer (model 14T, Thermo-Electron, Hopkinton, MA) was dedicated to continuous analysis of a stream drawn from midlevel of the chamber. The analyzer was calibrated daily using a gas-phase titration calibrator (model 101N or 102N, Thermo-Electron). The calibrator received nitric oxide from a tank, diluted it, and oxidized it using an ozone source to provide known concentrations of NO_2. Calibrations were checked against gases supplied from the National Bureau of Standards. Additional calibration checks were performed periodically by analyzing gas samples from the chamber by the Saltzman wet chemistry method (Saltzman 1954).

DIESEL EXHAUST GENERATION

Diesel exhaust was generated and characterized as done for previous long-term exhaust exposures at this Institute (McClellan et al. 1982), using methods described in the literature (Cheng et al. 1984; Mokler et al. 1984). Exhaust was generated by two 1980 Oldsmobile 5.7-liter engines used on alternate months. This engine type was selected to reproduce exposures, and the resulting health effects, that were obtained in previous studies at this Institute, and because the type represented the only U.S.-manufactured diesel engine being placed in passenger cars at the time the study was initiated. The engines were operated on the Federal Test Procedure urban driving cycle (U.S. Environmental Protection Agency 1977) with one exception. The driveshaft was not braked to a complete stop, as specified by the cycle, because of the complexity of the computer-dynamometer interaction that would have been required. The driveshaft turned at a simulated ground speed of approximately 3 to 5 mph with the engine at idle. The cycle operates a vehicle over 7.5 miles with an average speed of 19.5 mph in idle, acceleration, deceleration, and cruise modes.

A D-2 control fuel (Phillips Chemical Company, Bartlesville, OK) that meets EPA certification fuel specifications (U.S. Environmental Protection Agency 1977) was used. The lot of fuel used in this study had chemical and physical characteristics nearly identical to those listed for a lot used in studies reported previously (Cheng et al. 1984). Key characteristics were as follows: cetane number = 46, API gravity = 35, total sulfur = 0.35 percent, aromatics (FIA) = 32 volume percent, C/H ratio = 6.7, and antioxidant = 30 ptb DuPont FOA No. 11. The fuel was stored in underground bulk tanks, pumped to a holding tank in the engine room, and prefiltered before entering the engine's standard filtration and distribution system.

The engines were operated in an indoor, room-temperature environment, and intake air for combustion was HEPA-filtered (the same source as clean exposure-chamber supply air). The engines were maintained using lubricants, filters, and schedules specified in the 1980 Oldsmobile Service Manual (General Motors). The crankcase oil was SAE 30, API SE-CC (Heavy Duty Motor Oil, Amalie Refining Co., Div. of Witco Chemical Corp., Bradford, PA). Crankcase oil and filter were changed at 3,000-mile intervals. The engines were cooled by closed-loop heat exchangers. Engine coolant, a 50 percent glycol (antifreeze) mixture with water, was circulated normally using the engine's water pump, and was pressurized using a standard radiator cap to 14 psi. The other side of the closed-loop cooling system was tap-water regulated by a mechanical heat detector and valve to flow around engine coolant tubes in a single pass.

The engines were mounted on test stands and connected via three-speed automatic transmissions (200C, General Motors) to eddy-current dynamometers (A-300, Zollner, Kiel, Federal Republic of Germany) and inertial flywheels (Pohl Associates, Hatfield, PA). The dynamometers simulated resistive loads and the flywheels simulated inertial loads. The engine throttles were controlled by electromechanical actuators (DYNC 14000 Plus 4, Barber-Coleman, Rockford, IL). The throttle actuators and dynamometers were controlled by a microcomputer (LSI-11/2,

Digital Equipment Corp., Maynard, MA). Engine and driveshaft speeds were measured by magnetic pick-ups and compared by the computer to tables of desired speeds specified at one-second intervals. A servo-loop equation compared actual and desired speeds and computed the required throttle position. When the deceleration required was not achieved solely by releasing the throttle, additional voltages were applied to the dynamometer for braking. Computer and flywheel parameters were set to simulate an Oldsmobile Cutlass-class vehicle with a weight of 2,050 kg, a frontal area of 1.95 m^2, a rear axle ratio of 2.41, and a wheel-rolling radius of 31.8 cm. This class was typical of passenger cars using the 3.5-liter diesel engine during the model years 1979 to 1985.

Prior to its first use for exposures, each engine was operated for 3,000 miles on the Highway Fuel Economy Test Cycle (U.S. Environmental Protection Agency 1977) as a "break-in" period. Prior to each daily exposure, the engine was taken through a standardized warm-up procedure. It was started and idled for one minute, then operated through three Warm-Up Effects cycles, each identical to the first 125 seconds of the Federal Test Procedure cycle (U.S. Environmental Protection Agency 1977). The engine was operated through a single Highway Fuel Economy Test cycle of 764 seconds, and then idled until placed on the Federal Test Procedure cycle for exposures. Exposures were conducted with the engine operating on repeated cycles of 1,372 seconds each.

Exhaust was passed through a standard Oldsmobile Cutlass exhaust system, including muffler and tailpipe. At approximately 1 m beyond the normal discharge point, the entire exhaust was injected into a 35.6-cm inner diameter dilution tunnel and diluted 10:1 with clean, HEPA-filtered air under turbulent conditions. Approximately 5.5 m down the tunnel, a portion of the diluted exhaust was diverted into a serial dilution-distribution system for exposures. Excess exhaust was passed to the external atmosphere via a roof stack. The residence time for exhaust in the dilution tunnel was approximately one to two seconds before withdrawal for exposures.

MEASUREMENT OF EXHAUST EXPOSURE ATMOSPHERE

The exhaust atmosphere was measured as described previously (Cheng et al. 1984). Aerosol concentrations were measured by weighing filter samples. A single, seven-hour, integrated sample was drawn from the diesel exhaust and from the control chambers, in rotation with other chambers, at the same concentrations on each exposure day. Samples were collected on 25-mm glass-fiber filters (Type AE, Gelman, Ann Arbor, MI) from a midchamber sampling port at a sampling rate of 3 lpm. The filters, with approximately 4 to 5 mg of soot each, were weighed on a sensitive balance. The size distribution of the soot particles was determined using a Lovelace multiplejet impactor followed by a parallel-flow diffusion battery, as described in detail in the literature (Cheng et al. 1984).

Samples for gas analysis were taken weekly in Tedlar bags from a midchamber sampling port. The bags were identical to those used for automotive certification testing. The samples were analyzed for CO and carbon dioxide by infrared absorption (model 865, Beckman Instruments, Fullerton, CA), for vapor phase hydrocarbons by flame ionization (model 400, Beckman), and for nitrogen oxides, total N_2, and ammonia by chemiluminescence (model 14T, Thermo-Electron). The instruments were periodically calibrated by multipoint checks, and were calibrated by single-point checks before every measurement, using gases of known concentrations (National Bureau of Standards, Washington, DC). The chemical and biological (mutagenic) properties of the exhaust particles and their relationships to the Federal Test Procedure cycle have been reported previously (Bechtold et al. 1984).

APPENDIX C. Statistical Analyses

All data were filed and analyzed using a VAX 11/780 computer (Digital Equipment Corp., Maynard, MA).

PROCEDURES FOR ANALYSES OF DATA COLLECTED SERIALLY

The following procedures were used for data collected after 12, 18, and 24 months of exposure, including respiratory function, airway fluid enzymes, cytology, collagenous peptides, and lung morphometry. Body weight was measured both during respiratory function tests and when animals were killed for collection of morphometric data.

The data for each parameter measured serially were analyzed by three-way ANOVA to evaluate the significances of separate and interactive influences of emphysema, exposure (NO_2 or diesel exhaust), and time (Program BMDP2V, "Analysis of Variance and Covariance with Repeated Measures," Edition 1983, Version 1987, BMDP Statistical Software, Los Angeles, CA).

The data for each measurement time were analyzed for exposure-related and emphysema-related group differences by one-way ANOVA and multiple *t* tests (Program BMDP7D, "Description of Groups [Strata] with Histograms and Analysis of Variance," Edition 1983, Version 1987, BMDP Statistical

Software, Los Angeles, CA). Multiple comparisons of exposed versus control and emphysematous versus normal rats were performed separately for NO_2 and diesel exhaust groups. Four contrasts of each parameter were performed: normal exposed versus normal control rats; emphysematous exposed versus emphysematous control rats; emphysematous control versus normal control rats; and emphysematous exposed versus normal exposed rats. The Levene test for equality of variances (Levene 1960) was applied to all parameters, and demonstrated that the assumption of equal variances among the groups was seldom valid. The t values used to estimate significances of group differences were therefore derived using separate (rather than pooled) variances throughout. The Games method (Games 1977) was used to adjust critical t values for multiple comparisons. The criterion for statistical significance was set at $p < 0.05$ for these analyses and all those described below.

PROCEDURES FOR ANALYSES OF DATA COLLECTED ONLY ONCE

Data for lung collagen and immunological parameters were analyzed by two-way ANOVA, using the program described above, to evaluate the separate and interactive influences of emphysema and exposure. The analyses were performed separately for NO_2 and diesel exhaust.

The data for immune responses were analyzed using absolute values for optical density for serum IgM levels. Data for cell numbers were transformed to logs before analysis, because the variance of these data typically increases linearly with the mean level of response (Bice et al. 1985). The Levene test for equality of variance demonstrated that the variances of log-transformed data did not differ significantly among the groups.

The key parameter for clearance of inhaled radiolabeled particles was the half-time of the long-term ^{134}Cs clearance component. Two-component negative exponential functions were fit to the unweighted, whole-body radioactivity counts using a pseudo-Gauss-Newton algorithm (Program BMDPAR, "Derivative-Free Nonlinear Regression," Edition 1983, Version 1987, BMDP Statistical Software, Los Angeles, CA). Significances of differences among the second (long-term) clearance components of the various treatment groups were analyzed by calculating an F-statistic (Gallant 1975), followed by multiple comparisons using the Bonferroni adjustment of the critical F value.

APPENDIX D. Respiratory Function

Table D.1. Respiratory Function After 12 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 16)		Emphysema (E) (n = 16)		No Emphysema (N) (n = 16)		Emphysema (E + N) (n = 16)		No Emphysema (D) (n = 16)		Emphysema (E + D) (n = 16)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (BW)	g	373	29	381	26	386	20	387	31	387	29	390	24
Respiratory frequency	breaths/min	56	3	56	2	56	3	57	2	56	3	57	2
Tidal volume	ml	1.5	0.2	1.4	0.2	1.6	0.3	1.5	0.3	1.5	0.3	1.5	0.2
Minute volume (MV)	ml/min	83	12	79	10	90	15	83	12	84	14	83	11
MV/BW	ml/min/kg	223	29	208	28	233	39	215	26	218	43	214	32
Total lung capacity (TLC)	ml	15.8	1.5	19.3[a]	1.5	17.0[b]	1.2	20.8[a]	2.0	16.5	1.2	19.9[a]	1.4
TLC/BW	ml/kg	42	4	51[a]	4	44.3	2.7	53.5[a]	5.0	43	3	51[a]	4
Vital capacity (VC)	ml	14.3	1.2	16.8[a]	1.2	15.4[b]	1.2	17.7[a]	1.4	14.8	1.2	17.4[a]	1.5
VC/TLC	%	91	3	88	4	91	2	88[a]	4	90	3	87	4
Functional residual capacity (FRC)	ml	2.7	0.3	4.5[a]	0.7	2.9	0.3	4.6[a]	0.8	3.0	0.4	4.2[a]	0.6
FRC/TLC	%	17	2	23[a]	3	17	2	22[a]	3	18	2	21[a]	3
Residual volume (RV)	ml	1.5	0.6	2.4	0.9	1.6	0.4	2.9[a]	0.9	1.7	0.5	2.5[a]	0.8
RV/TLC	%	9	3	12	4	10	2	14[a]	4	10	3	13	4
Total pulmonary resistance	cm H_2O/ml/sec	0.13	0.04	0.12	0.04	0.12	0.03	0.11	0.04	0.13	0.04	0.13	0.04
Dynamic lung compliance (Cdyn)	ml/cm H_2O	0.56	0.15	0.62	0.15	0.55	0.07	0.77[a,b]	0.16	0.49	0.09	0.73[a]	0.23
Cdyn/FRC	ml/cm H_2O/ml	0.21	0.05	0.14[a]	0.04	0.19	0.04	0.17	0.05	0.17[b]	0.03	0.17	0.05
Quasistatic chord compliance (Cqs)	ml/cm H_2O	1.00	0.12	1.14[a]	0.15	1.07	0.10	1.21[a]	0.14	1.01	0.10	1.21[a]	0.13
Cqs/FRC	ml/cm H_2O/ml	0.37	0.04	0.26[a]	0.05	0.37	0.06	0.27[a]	0.06	0.34	0.04	0.29[a]	0.06
CO diffusing capacity (DL_{CO})	ml/min/mm Hg	0.319	0.028	0.306	0.036	0.357[b]	0.045	0.348[b]	0.050	0.334	0.044	0.339	0.039
DL_{CO}/lung volume	ml/min/mm Hg/ml	0.020	0.0002	0.016[a]	0.002	0.021	0.003	0.017[a]	0.002	0.021	0.002	0.017[a]	0.002
Forced vital capacity (FVC)	ml	15.1	1.5	17.6[a]	1.4	16.4[b]	1.3	18.6[a]	1.6	15.7	1.3	18.2[a]	1.6
FVC/BW	ml/kg	41	5	46[a]	4	43	3	48[a]	4	41	3	47[a]	5
% FVC exhaled in 0.1 second	%	62	7	47[a]	6	59	7	47[a]	3	61	8	48[a]	7
Peak expiratory flow rate (PEFR)	ml/sec	132	11	118[a]	13	132	8	119[a]	10	133	9	118[a]	11
PEFR/FVC	ml/sec/ml	8.8	1.0	6.8[a]	1.0	8.1	0.7	6.4[a]	0.5	8.5	0.7	6.5[a]	0.9
% FVC at PEFR	%	74	8	85[a]	5	75	7	86[a]	3	72	9	85[a]	5
Mean midexpiratory flow (MMEF)	ml/sec	80	18	54[a]	9	84	19	56[a]	10	83	24	55[a]	12
MMEF/FVC	ml/sec/ml	5.3	1.2	3.1[a]	0.6	5.1	1.1	3.0[a]	0.5	5.3	1.5	3.0[a]	0.7
Flow difference at 25% FVC (concavity)	ml/sec	−6.4	6.9	−1.4	7.0	9.2	7.7	4.0[a]	5.8	−5.0	6.6	−0.6	6.3
Expiratory flow at 50% FVC (F_{50})	ml/sec	84	19	59[a]	10	86	21	62[a]	13	89	28	59[a]	14
F_{50}/FVC	ml/sec/ml	5.6	1.3	3.3[a]	0.6	5.3	1.2	3.4[a]	0.7	5.6	1.6	3.3[a]	0.8
Expiratory flow at 25% FVC (F_{25})	ml/sec	49	12	31[a]	8	52	13	27[a]	8	49	14	30[a]	9
F_{25}/FVC	ml/sec/ml	3.2	0.7	1.8[a]	0.5	3.2	0.7	1.5[a]	0.4	3.1	0.9	1.7[a]	0.5
Expiratory flow at 10% FVC (F_{10})	ml/sec	20	4	11[a]	4	21	5	9[a]	3	21	5	10[a]	3
F_{10}/FVC	ml/sec/ml	1.3	0.3	0.6[a]	0.2	1.3	0.3	0.5[a]	0.1	1.3	0.2	0.6[a]	0.2
Slope of phase III of N_2 washout	% N_2/ml	0.43	0.15	0.45	0.12	0.37	0.11	0.46	0.17	0.44	0.15	0.39	0.15

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

Table D.2. Respiratory Function After 18 Months of Exposure

Parameter	Unit	Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 16)		Emphysema (E) (n = 16)		No Emphysema (N) (n = 16)		Emphysema (E + N) (n = 16)		No Emphysema (D) (n = 16)		Emphysema (E + D) (n = 16)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight	g	385	28	374	29	393	20	380	26	386	28	372	31
Respiratory frequency	breaths/min	55	4	55	4	57	4	57	3	57	3	55	4
Tidal volume	ml	1.3	0.2	1.4	0.2	1.4	0.2	1.4	0.2	1.3	0.2	1.4	0.3
Minute volume	ml/min	75	11	78	12	77	12	79	10	71	12	75	12
MV/BW	ml/min/kg	194	24	210	40	196	31	210	36	186	37	202	28
Total lung capacity	ml	15.9	1.5	19.6[a]	1.3	17.5	1.4	21.7[a,b]	2.8	16.1	1.2	19.5[a]	2.4
TLC/BW	ml/kg	41	4	53[a]	6	45[b]	3	57[a,b]	8	42	2	53[a]	6
Vital capacity	ml	14.2	1.2	17.0[a]	1.2	15.4	1.1	18.7[a]	2.1	14.2	1.1	16.9[a]	2.0
VC/TLC	%	90	3	87	3	88	4	86	4	88	3	87	3
Functional residual capacity	ml	2.9	0.4	4.7[a]	0.7	3.4	0.5	5.3	1.4	3.2	0.4	4.7[a]	1.0
FRC/TLC	%	19	2	24[a]	2	19	2	24[a]	4	20	2	24[a]	4
Residual volume	ml	1.7	0.5	2.6	0.7	2.0	0.8	3.0[a]	1.1	2.0	0.4	2.6[a]	0.7
RV/TLC	%	10	3	13	3	12	4	14	3	12	2	13	3
Total pulmonary resistance	cm H_2O/ml/sec	0.16	0.04	0.13	0.03	0.16	0.06	0.12	0.03	0.12[b]	0.04	0.13	0.04
Dynamic lung compliance	ml/cm H_2O	0.50	0.13	0.88[a]	0.22	0.55	0.11	1.08[b]	0.35	0.56	0.13	0.79[a]	0.23
Cdyn/FRC	ml/cm H_2O/ml	0.17	0.05	0.19[a]	0.04	0.17	0.04	0.20[a]	0.04	0.17	0.04	0.17	0.05
Quasistatic chord compliance	ml/cm H_2O	0.99	0.13	1.13[a]	0.11	1.06	0.11	1.22[a]	0.12	0.95[b]	0.11	1.12[a]	0.14
Cqs/FRC	ml/cm H_2O/ml	0.34	0.05	0.24[a]	0.03	0.32	0.05	0.24[a]	0.06	0.30[b]	0.04	0.25[a]	0.05
CO diffusing capacity	ml/min/mm Hg	0.304	0.022	0.291	0.033	0.328	0.042	0.305	0.046	0.283	0.043	0.303	0.039
DL_{CO}/lung volume	ml/min/mm Hg/ml	0.019	0.002	0.015[a]	0.002	0.019	0.002	0.014	0.002	0.017[b]	0.002	0.015[a]	0.002
Forced vital capacity	ml	14.9	1.1	17.6[a]	1.2	16.1[b]	1.1	19.3[a,b]	2.0	15.0	1.3	17.7[a]	1.9
FVC/BW	ml/kg	39	4	48[a]	6	41	2	51[a]	6	39	2	48[a]	5
% FVC exhaled in 0.1 second	%	83	8	47[a]	5	58	6	44[a]	6	64	6	47[a]	4
Peak expiratory flow rate	ml/sec	124	6	113[a]	7	123	8	111[a]	11	126	3	115[a]	8
PEFR/FVC	ml/sec/ml	8.4	0.7	6.5[a]	0.6	7.7[b]	0.6	5.8[a,b]	0.8	8.5	0.7	6.6[a]	0.7
% FVC at PEFR	%	71	7	84[a]	4	72	7	84[a]	6	68	6	84[a]	4
Mean midexpiratory flow	ml/sec	84	19	55[a]	10	80	15	53[a]	16	87	13	56[a]	8
MMEF/FVC	ml/sec/ml	5.7	1.3	3.1[a]	0.6	4.9	0.9	2.8[a]	1.0	5.9	1.1	3.2[a]	0.5
Flow difference at 25% FVC (concavity)	ml/sec	−5.0	5.6	0.8[a]	5.1	−4.7	6.6	2.4[a]	6.9	−6.3	5.2	−0.3[a]	5.5
Expiratory flow at 50% FVC	ml/sec	90	21	61[a]	11	85	18	58[a]	16	93	18	60[a]	9
F_{50}/FVC	ml/sec/ml	6.0	1.4	3.5[a]	0.7	5.2	1.1	3.1[a]	1.0	6.3	1.5	3.4[a]	0.6
Expiratory flow at 25% FVC	ml/sec	50	13	30[a]	7	47	7	27[a]	11	53	9	31[a]	7
F_{25}/FVC	ml/sec/ml	3.4	0.9	1.7[a]	0.4	2.9	0.4	1.4[a]	0.7	3.6	0.7	1.7[a]	0.4
Expiratory flow at 10% FVC	ml/sec	17	3	9[a]	3	18	3	10[a]	4	19	5	9[a]	2
F_{10}/FVC	ml/sec/ml	1.2	0.2	0.5[a]	0.2	1.1	0.2	0.5[a]	0.3	1.3	0.3	0.5[a]	0.1
Slope of phase III of N_2 washout	% N_2/ml	0.48	0.10	0.54	0.07	0.49	0.27	0.52	0.15	0.52	0.10	0.50	0.14

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

Table D.3. Respiratory Function After 24 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 14)		Emphysema (E) (n = 16)		No Emphysema (N) (n = 16)		Emphysema (E + N) (n = 16)		No Emphysema (D) (n = 16)		Emphysema (E + D) (n = 16)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight	g	369	31	353	35	359	38	362	16	371	30	356	42
Respiratory frequency	breaths/min	54	4	55	5	55	9	56	4	55	4	55	4
Tidal volume	ml	1.6	0.2	1.4	0.3	1.5	0.3	1.4	0.3	1.5	0.3	1.6	0.3
Minute volume	ml/min	87	14	76	12	78	15	77	14	83	16	87	13
MV/BW	ml/min/kg	236	39	218	40	218	38	211	32	220	35	250	58
Total lung capacity	ml	17.2	1.7	20.3[a]	2.3	18.3	1.2	23.6[a,b]	2.3	16.0	1.2	21.1[a]	2.2
TLC/BW	ml/kg	47	6	58[a]	8	52	8	65[a,b]	7	43	4	60[a]	11
Vital capacity	ml	15.2	1.4	17.3[a]	2.1	16.2	1.0	20.1[a,b]	2.0	14.3	1.3	17.9[a]	2.2
VC/TLC	%	89	2	86[a]	3	88	2	85[a]	2	89	3	85[a]	4
Functional residual capacity	ml	3.5	0.6	5.6[a]	0.9	3.8	0.5	6.4[a]	1.0	3.5	0.5	6.0[a]	1.2
FRC/TLC	%	20	2	28[a]	4	21	2	27[a]	4	22	4	28[a]	4
Residual volume	ml	2.0	0.5	2.9[a]	0.6	2.1	0.5	3.5[a]	0.6	1.8	0.4	3.2[a]	0.9
RV/TLC	%	11	2	15[a]	3	12	2	15[a]	2	11	3	15[a]	4
Total pulmonary resistance	cm H_2O/ml/sec	0.13	0.06	0.11	0.06	0.13	0.03	0.13	0.05	0.12	0.04	0.10	0.04
Dynamic lung compliance	ml/cm H_2O	0.70	0.18	1.14[a]	0.25	0.86	0.22	1.27[b]	0.23	0.67	0.17	1.15[a]	0.29
Cdyn/FRC	ml/cm H_2O/ml	0.21	0.06	0.21	0.05	0.23	0.05	0.20[a]	0.03	0.19	0.06	0.20	0.05
Quasistatic chord compliance	ml/cm H_2O	1.03	0.12	1.12[a]	0.19	1.10	0.10	1.25[a]	0.16	0.94	0.12	1.13[a]	0.13
Cqs/FRC	ml/cm H_2O/ml	0.30	0.05	0.20[a]	0.04	0.29	0.05	0.20[a]	0.03	0.27	0.05	0.20[a]	0.04
CO diffusing capacity	ml/min/mm Hg	0.265	0.041	0.250	0.078	0.288	0.085	0.312[b]	0.045	0.254	0.047	0.269	0.057
DL_{CO}/lung volume	ml/min/mm Hg/ml	0.016	0.003	0.012[a]	0.004	0.016	0.005	0.014	0.003	0.016	0.003	0.013[a]	0.003
Forced vital capacity	ml	15.9	1.5	17.7[a]	2.2	16.9	1.2	20.7[a,b]	2.0	14.8	1.4	18.3[a]	2.3
FVC/BW	ml/kg	43	4	51[a]	8	48	8	57[a]	6	40	4	52[a]	11
% FVC exhaled in 0.1 second	%	59	8	48[a]	5	58	3	41[a,b]	5	67[b]	5	46[a]	9
Peak expiratory flow rate	ml/sec	127	8	116[a]	7	126	6	117	11	128	3	112[a]	15
PEFR/FVC	ml/sec/ml	8.0	0.9	6.6[a]	0.9	7.4	0.6	5.7[a,b]	0.5	8.7	0.8	6.2[a]	1.2
% FVC at PEFR	%	71	6	83[a]	3	72	4	83[a]	6	67	5	83[a]	6
Mean midexpiratory flow	ml/sec	82	18	55[a]	9	86	12	53[a]	5	97[b]	11	58[a]	12
MMEF/FVC	ml/sec/ml	5.2	1.3	3.2[a]	0.8	5.1	0.6	2.6[a]	0.5	6.6[b]	1.0	3.3[a]	1.0
Flow difference at 25% FVC (concavity)	ml/sec	−3.2	7.1	1.5	4.7	−3.9	5.0	2.2[a]	4.1	−4.5	6.6	3.1[a]	5.6
Expiratory flow at 50% FVC	ml/sec	87	22	59[a]	9	92	14	55[a]	10	107[b]	15	65[a]	13
F_{50}/FVC	ml/sec/ml	5.6	1.6	3.4[a]	0.7	5.4	0.7	2.7[a]	0.8	7.2[b]	1.2	3.7[a]	1.1
Expiratory flow at 25% FVC	ml/sec	47	11	28[a]	6	50	8	24[a]	7	58[b]	8	29[a]	9
F_{25}/FVC	ml/sec/ml	3.0	0.8	1.6[a]	0.6	3.0	0.4	1.2[a,b]	0.4	3.9[b]	0.6	1.7[a]	0.7
Expiratory flow at 10% FVC	ml/sec	16	4	10[a]	3	19	3	7[a,b]	3	21[b]	3	10[a]	4
F_{10}/FVC	ml/sec/ml	1.0	0.4	0.6[a]	0.2	1.1	0.2	0.4[a,b]	0.2	1.4[b]	0.2	0.6[a]	0.3
Slope of phase III of N_2 washout	% N_2/ml	0.43	0.07	0.47	0.10	0.38	0.07	0.43	0.09	0.49	0.11	0.45	0.07

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

Table D.4. Significant Influences of Nitrogen Dioxide, Emphysema, and Time on Respiratory Function

	Significant Single Influence			Significant Interactions			
Parameter	Emphysema	Nitrogen Dioxide	Time	Emphysema + Nitrogen Dioxide	Emphysema + Time	Nitrogen Dioxide + Time	Emphysema + Nitrogen Dioxide + Time
Body weight			+				
Respiratory frequency		+					
Tidal volume			+				
Minute volume			+				
MV/BW			+		+		
Total lung capacity	+	+	+				
TLC/BW	+	+	+				
Vital capacity	+	+	+				
VC/TLC	+		+				
Functional residual capacity	+	+	+		+		
FRC/TLC	+		+				
Residual volume	+	+	+				
RV/TLC	+		+				
Total pulmonary resistance	+		+				
Dynamic lung compliance Cdyn/FRC	+						
Quasistatic chord compliance	+		+				
Cqs/FRC	+		+				
CO diffusing capacity		+	+				
DL_{CO}/lung volume	+		+				
Forced vital capacity	+	+	+				
FVC/BW	+	+	+				
% FVC exhaled in 0.1 second	+	+					
Peak expiratory flow rate	+		+				
PEFR/FVC	+	+	+				
% FVC at PEFR	+						
Mean midexpiratory flow	+						
MMEF/FVC	+	+					
Flow difference at 25% FVC (concavity)	+			+			
Expiratory flow at 50% FVC	+						
F_{50}/FVC	+	+					
Expiratory flow at 25% FVC	+						
F_{25}/FVC	+	+					
Expiratory flow at 10% FVC	+		+	+			
F_{10}/FVC	+		+				
Slope of phase III of N_2 washout	+		+				

Table D.5. Significant Influences of Diesel Exhaust, Emphysema, and Time on Respiratory Function

Parameter	Significant Single Influence			Significant Interactions			
	Emphysema	Diesel Exhaust	Time	Emphysema + Diesel Exhaust	Emphysema + Time	Diesel Exhaust + Time	Emphysema + Diesel Exhaust + Time
Body weight			+				
Respiratory frequency							
Tidal volume			+				
Minute volume			+				
MV/BW			+				
Total lung capacity	+		+				
TLC/BW	+		+		+		
Vital capacity	+						
VC/TLC	+		+				
Functional residual capacity	+		+		+		
FRC/TLC	+		+	+			
Residual volume	+		+				
RV/TLC	+		+				
Total pulmonary resistance			+				
Dynamic lung compliance	+						
Cdyn/FRC							
Quasistatic chord compliance	+						
Cqs/FRC	+	+	+				
CO diffusing capacity			+	+			
DL_{CO}/lung volume	+		+	+			
Forced vital capacity	+						
FVC/BW	+		+				
% FVC exhaled in 0.1 second	+						+
Peak expiratory flow rate	+		+				
PEFR/FVC	+						
% FVC at PEFR	+		+				
Mean midexpiratory flow	+	+					
MMEF/FVC	+					+	
Flow difference at 25% FVC							
(concavity)	+		+				
Expiratory flow at 50% FVC	+	+					
F_{50}/FVC	+	+	+			+	
Expiratory flow at 25% FVC	+						
F_{25}/FVC	+						
Expiratory flow at 10% FVC	+	+	+	+			
F_{10}/FVC	+	+		+		+	
Slope of phase III of N_2							
washout			+	+			

APPENDIX E. Cytology and Chemistry of Airway Fluid

Table E.1. Airway Fluid Constituents After 12 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 8)		Emphysema (E) (n = 8)		No Emphysema (N) (n = 8)		Emphysema (E + N) (n = 8)		No Emphysema (D) (n = 8)		Emphysema (E + D) (n = 8)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total leukocytes	10^3 cells/ml	772	306	823	463	509	255	1066[a]	459	486	299	879	370
Macrophages	10^3 cells/ml	520	271	542	308	387	184	781	411	272	168	550[a]	218
Neutrophils	10^3 cells/ml	110	156	175	114	51	46	114	80	110	87	168	123
Total protein	mg/ml	0.37	0.10	0.32	0.07	0.21[b]	0.05	0.23[b]	0.05	0.32	0.11	0.24	0.15
Sialic acid	nmol/ml	17.2	4.4	14.5	2.9	12.3[b]	1.5	12.4	3.9	12.2[b]	2.9	12.0	3.0
Lactate dehydrogenase	mIU/ml	71	20	90	24	85	32	94	21	100[b]	15	104	24
β-Glucuronidase	mIU/ml	0.10	0.05	0.25	0.17	0.11	0.05	0.16	0.06	0.46[b]	0.17	0.29	0.14
Alkaline phosphatase	mIU/ml	29	6	28	5	34	8	30	11	32	7	33	7
Glutathione reductase	mIU/ml	7.4	1.5	9.3	1.8	9.1	2.2	10.0	2.1	8.7	1.6	9.0	1.3
Glutathione peroxidase	mIU/ml	2.2	1.2	2.9	1.6	2.5	1.4	2.6	1.4	2.6	1.4	2.5	1.3
Acid proteinase	activity/ml[c]	42	6	50[a]	5	43	7	43[b]	5	80[b]	12	62[a,b]	8
Cathepsin B	mIU/ml	0.032	0.007	0.045	0.012	0.025	0.007	0.031[b]	0.005	0.044	0.011	0.037	0.009
Collagenous peptides	μg/ml	4.7	0.8	5.0	1.2	4.4	0.7	5.8	1.3	5.0	0.5	4.4	0.6

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

[c] Acid proteinase activity: micrograms of hemoglobin solubilized in four hours.

Table E.2. Airway Fluid Constituents After 18 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 8)		Emphysema (E) (n = 7)		No Emphysema (N) (n = 8)		Emphysema (E + N) (n = 8)		No Emphysema (D) (n = 8)		Emphysema (E + D) (n = 6)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total leukocytes	10^3 cells/ml	290	142	473	197	221	113	278	104	227	94	323	164
Macrophages	10^3 cells/ml	245	108	383	155	185	95	238	98	95[a]	55	168[a,b]	37
Neutrophils	10^3 cells/ml	4	9	6	9	7	6	8	7	106[a]	61	104	100
Total protein	mg/ml	0.29	0.13	0.39	0.12	0.29	0.11	0.33	0.11	0.55[a]	0.16	0.33[b]	0.10
Sialic acid	nmol/ml	7.5	3.9	10.8	6.4	9.9	3.5	12.1	8.0	16.5[a]	6.3	8.6[b]	2.1
Lactate dehydrogenase	mIU/ml	76	36	120	54	95	54	137	43	195[a]	60	141	53
β-Glucuronidase	mIU/ml	0.38	0.18	0.52	0.24	0.43	0.18	0.40	0.10	1.56[a]	0.69	0.87	0.50
Alkaline phosphatase	mIU/ml	36	16	35	18	39	17	33	6	58	27	56	19
Glutathione reductase	mIU/ml	11.2	2.6	14.5	4.6	15.4[a]	2.5	16.9	3.8	18.0[a]	3.3	17.2	3.8
Glutathione peroxidase	mIU/ml	3.5	1.5	4.3	1.2	4.2	1.1	5.5	2.9	6.3[a]	0.6	4.3[b]	1.0
Acid proteinase	activity/ml[c]	52	6	67	12	56	13	60	9	85[a]	30	91[a]	17
Cathepsin B	mIU/ml	0.064	0.017	0.117	0.057	0.075	0.038	0.068	0.014	0.164[a]	0.095	0.182	0.089
Collagenous peptides	μg/ml	4.9	0.9	5.8	1.5	5.2	1.2	5.5	1.5	7.7[c]	1.7	5.7[b]	0.8

[a] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[c] Acid proteinase activity: micrograms of hemoglobin solubilized in four hours.

Table E.3. Airway Fluid Constituents After 24 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 8)		Emphysema (E) (n = 7)		No Emphysema (N) (n = 8)		Emphysema (E + N) (n = 8)		No Emphysema (D) (n = 8)		Emphysema (E + D) (n = 8)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total leukocytes	10^3 cells/ml	145	42	167	72	109	41	180	99	128	47	132	82
Macrophages	10^3 cells/ml	123	40	140	57	104	39	164	84	59[a]	24	85	42
Neutrophils	10^3 cells/ml	1	1	0	0	1	3	8	16	52[a]	16	28[a]	24
Total protein	mg/ml	0.14	0.04	0.18	0.08	0.26[a]	0.09	0.29	0.11	0.37[a]	0.08	0.29	0.10
Sialic acid	nmol/ml	7.9	1.9	10.7	7.2	11.8	4.5	12.6	4.5	19.0[a]	4.9	10.5[b]	4.2
Lactate dehydrogenase	mIU/ml	74	28	102	58	143[a]	56	225[a]	96	311[a]	86	154[b]	40
β-Glucuronidase	mIU/ml	0.27	0.14	0.47	0.19	0.34	0.11	0.54	0.28	3.29[a]	0.71	1.23[a,b]	0.41
Alkaline phosphatase	mIU/ml	20	5	17	7	32	13	34	21	57[a]	20	30[b]	11
Glutathione reductase	mIU/ml	6.0	2.8	9.6	4.6	9.5	5.9	15.4	4.6	13.5[a]	3.8	9.3[b]	1.6
Glutathione peroxidase	mIU/ml	1.0	1.0	1.2	0.6	0.6	0.6	2.2	1.8	2.8[a]	1.1	2.5	1.5
Acid proteinase	activity/ml[c]	32	6	47	18	36	10	42	12	88[a]	13	67[a,b]	7
Cathepsin B	mIU/ml	0.022	0.010	0.032	0.019	0.027	0.013	0.037	0.020	0.063[a]	0.029	0.046	0.025
Collagenous peptides	μg/ml	4.1	0.6	3.9	1.3	5.0	1.0	6.4[a]	1.9	10.4[a]	2.4	7.6[a]	3.1

[a] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[c] Acid proteinase activity: micrograms of hemoglobin solubilized in four hours.

Table E.4. Significant Influences of Nitrogen Dioxide, Emphysema, and Time on Airway Fluids

	Significant Single Influences			Significant Interactions			
Parameter	Emphysema	Nitrogen Dioxide	Time	Emphysema + Nitrogen Dioxide	Emphysema + Time	Nitrogen Dioxide + Time	Emphysema + Nitrogen Dioxide + Time
Total leukocytes	+		+				+
Macrophages	+		+				
Neutrophils			+				
Total protein			+			+	
Sialic acid			+			+	
Lactate dehydrogenase	+	+	+			+	
β-Glucuronidase	+		+				
Alkaline phosphatase		+	+				
Glutathione reductase	+	+	+				
Glutathione peroxidase	+		+				
Acid proteinase	+		+	+			
Cathepsin B	+		+	+			+
Collagenous peptides	+	+				+	

Table E.5. Significant Influences of Diesel Exhaust, Emphysema, and Time on Airway Fluids

	Significant Single Influences			Significant Interactions			
Parameter	Emphysema	Diesel Exhaust	Time	Emphysema + Diesel Exhaust	Emphysema + Time	Diesel Exhaust + Time	Emphysema + Diesel Exhaust + Time
Total leukocytes	+		+				
Macrophages	+	+	+				
Neutrophils		+	+			+	
Total protein	+	+	+	+		+	
Sialic acid	+		+	+		+	+
Lactate dehydrogenase	+	+	+	+	+	+	+
β-Glucuronidase	+	+	+	+	+	+	+
Alkaline phosphatase		+	+			+	
Glutathione reductase		+	+	+		+	
Glutathione peroxidase		+	+	+		+	
Acid proteinase		+	+	+	+		
Cathepsin B		+	+			+	
Collagenous peptides	+	+	+	+		+	

APPENDIX F. Total Lung Collagen After 24 Months of Exposure

Table F.1. Effects of Exposure on Total Lung Collagen

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 8)		Emphysema (E) (n = 7)		No Emphysema (N) (n = 8)		Emphysema (E + N) (n = 8)		No Emphysema (D) (n = 8)		Emphysema (E + D) (n = 8)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total lung collagen	mg	22.5	3.2	35.7[a]	7.1	31.8[b]	3.8	42.5[a]	7.5	32.4[b]	4.8	32.5	4.3
Lung weight	g	1.5	0.3	2.1	0.6	1.8	0.3	2.5	1.0	2.0	0.5	1.7	0.2
Collagen/lung weight	mg/g	15.6	4.6	18.6	7.4	17.8	2.4	18.6	5.3	16.5	2.9	19.0	2.5

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

[b] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

Table F.2. Significant Influences of Exposure and Emphysema on Total Lung Collagen

	Nitrogen Dioxide and Control			Diesel Exhaust and Control		
Parameter	Emphysema	Nitrogen Dioxide	Emphysema + Nitrogen Dioxide	Emphysema	Diesel Exhaust	Emphysema + Diesel Exhaust
Total lung collagen	+	+		+		+
Lung weight	+					+
Collagen/lung weight	+	+				

APPENDIX G. Lung Morphometry

Table G.1. Lung Morphometry After 12 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 7)		Emphysema (E) (n = 7)		No Emphysema (N) (n = 6)		Emphysema (E + N) (n = 8)		No Emphysema (D) (n = 6)		Emphysema (E + D) (n = 8)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight	g	369	19	391	21	375	22	391	28	388	15	378	22
Lung weight (LW)	g	1.13	0.15	1.20	0.13	1.13	0.25	1.29	0.27	1.20	0.22	1.27	0.21
LW/BW	g/kg	3.1	0.4	3.1	0.3	3.1	0.6	3.3	0.7	3.2	0.5	3.4	0.5
External lung volume (LV)	ml	12.0	1.2	17.2[a]	1.6	12.3	1.9	17.8[a]	1.7	12.5	1.0	16.4[a]	2.5
LV/BW	ml/kg	33	3	44[a]	4	33	5	46[a]	5	32	4	43[a]	6
LW/LV	g/ml	0.10	0.02	0.07	0.01	0.09	0.01	0.07	0.01	0.10	0.01	0.08	0.02
Mean linear intercept	µm	93	6	161[a]	6	91	9	165[a]	15	92	5	163[a]	11
Internal surface area	m^2	0.51	0.06	0.43[a]	0.04	0.54	0.10	0.44	0.06	0.55	0.07	0.40[a]	0.07

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by ANOVA and multiple comparison.

Table G.2. Lung Morphometry After 18 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 7)		Emphysema (E) (n = 7)		No Emphysema (N) (n = 8)		Emphysema (E + N) (n = 7)		No Emphysema (D) (n = 7)		Emphysema (E + D) (n = 6)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight	g	383	22	377	19	385	24	372	33	385	28	349	24
Lung weight	g	1.07	0.22	1.43[a]	0.16	1.28	0.16	1.62[a]	0.27	1.45	0.34	1.54	0.24
LW/BW	g/kg	2.8	0.6	3.8[a]	0.5	3.3	0.3	4.4[a]	0.7	3.8	0.8	4.4[a]	0.4
External lung volume	ml	13.1	0.7	18.5[a]	3.5	13.7	1.4	16.9[a]	1.4	13.7	1.7	17.2[a]	2.3
LV/BW	ml/kg	34	4	49[a]	10	36	3	45[a]	4	36	4	49[a]	5
LW/LV	g/ml	0.08	0.02	0.08	0.02	0.09	0.01	0.10	0.01	0.11	0.03	0.09	0.02
Mean linear intercept	µm	95	5	162[a]	7	98	4	155[a]	13	93	7	163[a]	9
Internal surface area	m^2	0.55	0.03	0.46[a]	0.08	0.56	0.05	0.44[a]	0.06	0.59	0.06	0.42[a]	0.06

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by ANOVA and multiple comparison.

Table G.3. Lung Morphometry After 24 Months of Exposure

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 8)		Emphysema (E) (n = 5)		No Emphysema (N) (n = 8)		Emphysema (E + N) (n = 6)		No Emphysema (D) (n = 8)		Emphysema (E + D) (n = 6)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight	g	371	20	370	32	359	17	353	24	367	34	362	39
Lung weight	g	1.52	0.35	1.77	0.44	1.80	0.25	2.00	0.35	2.02	0.49	1.75	0.18
LW/BW	g/kg	4.1	0.9	4.8	1.3	5.1	1.0	5.6	0.9	5.5	1.4	4.9	0.6
External lung volume	ml	15.5	2.9	20.8[a]	0.9	15.7	0.8	23.4[a]	2.9	14.1	2.2	20.1[a]	3.6
LV/BW	ml/kg	42	9	56[a]	5	44	3	66[a]	7	39	6	56[a]	10
LW/LV	g/ml	0.10	0.03	0.09	0.02	0.12	0.02	0.09[a]	0.02	0.15	0.05	0.09[a]	0.01
Mean linear intercept	µm	103	7	186[a]	9	103	7	176[a]	12	106	5	178[a]	17
Internal surface area	m^2	0.60	0.10	0.45[a]	0.02	0.61	0.06	0.53	0.07	0.53	0.07	0.45	0.06

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by ANOVA and multiple comparison.

Table G.4. Significant Influences of Nitrogen Dioxide, Emphysema, and Time on Lung Morphometry

	Significant Single Influences			Significant Interactions			
Parameter	Emphysema	Nitrogen Dioxide	Time	Emphysema + Nitrogen Dioxide	Emphysema + Time	Nitrogen Dioxide + Time	Emphysema + Nitrogen Dioxide + Time
Body weight			+				
Lung weight	+	+	+				
LW/BW	+	+	+		+		
External lung volume	+		+				
LV/BW	+		+				
LW/LV	+	+	+			+	
Mean linear intercept	+		+		+		
Internal surface area	+		+				

Table G.5. Significant Influences of Diesel Exhaust, Emphysema, and Time on Lung Morphometry

	Significant Single Influences			Significant Interactions			
Parameter	Emphysema	Diesel Exhaust	Time	Emphysema + Diesel Exhaust	Emphysema + Time	Diesel Exhaust + Time	Emphysema + Diesel Exhaust + Time
Body weight			+				
Lung weight		+	+	+			
LW/BW		+	+				
External lung volume	+		+				
LV/BW	+		+				
LW/LV	+	+	+	+			
Mean linear intercept	+		+				
Internal surface area	+						

APPENDIX H. Clearance of Radiolabeled Particles

Table H.1. Half-Time of the Second (Long-Term) Component of Clearance of ^{134}Cs-Labeled Fused Aluminosilicate Particles[a]

		Control				Nitrogen Dioxide				Diesel Exhaust			
		No Emphysema (C) (n = 7)		Emphysema (E) (n = 10)		No Emphysema (N) (n = 9)		Emphysema (E + N) (n = 9)		No Emphysema (D) (n = 9)		Emphysema (E + D) (n = 9)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Clearance half-time	days	52	8	59	8	51	5	58	13	109[b]	9	82[b,c]	13

[a] Rats inhaled radiolabeled particles after 18 months of exposure. Whole-body radioactivity was then counted serially for 120 days while the chronic exposures continued.

[b] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison.

[c] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by multiple comparison.

Table H.2. Significant Influences of Exposure and Emphysema on the Half-Time of Long-Term Clearance

	Nitrogen Dioxide and Control			Diesel Exhaust and Control		
Parameter	Emphysema	Nitrogen Dioxide	Emphysema + Nitrogen Dioxide	Emphysema	Diesel Exhaust	Emphysema + Diesel Exhaust
Clearance half-time	+			+	+	+

APPENDIX I. Lung Burdens of Diesel Soot

Table I.1. Lung Burdens of Diesel Soot

		12 Months				18 Months				24 Months			
		No Emphysema (n = 8)		Emphysema (n = 8)		No Emphysema (n = 8)		Emphysema (n = 8)		No Emphysema (n = 8)		Emphysema (n = 8)	
Parameter	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight	g	377	27	378	22	379	32	353	25	367	34	348	49
Lung weight	g	1.65	0.19	1.63	0.15	1.51	0.37	1.57	0.25	2.02	0.49	1.95	0.40
Soot lung burden	mg	3.84	0.81	1.50[a]	0.18	6.86	1.51	2.45[a]	0.81	12.13	1.71	4.48[a]	1.59
Soot lung burden/BW	mg/g	2.32	0.42	0.93[a]	0.17	4.86	1.76	1.62[a]	0.72	6.23	1.25	2.49[a]	1.17
Soot lung burden/LW	mg/kg	10.1	1.9	4.0[a]	0.7	18.3	4.3	7.0[a]	2.5	33.1	3.8	13.0[a]	4.6

[a] Difference from corresponding nonemphysematous group significant at $p < 0.05$ by t test.

APPENDIX J. Pulmonary Immune Responses After 24 Months of Exposure to Diesel Exhaust

Table J.1. Immune Responses to Intratracheally Instilled Sheep Red Blood Cells After 24 Months of Exposure

	Control				Diesel Exhaust			
	No Emphysema (C) (n = 6)		Emphysema (E) (n = 10)		No Emphysema (D) (n = 8)		Emphysema (E + D) (n = 5)	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Responses in Pulmonary Lymph Nodes								
Total cells (10^6 cells)	4.25	1.72	4.92	1.68	18.12[a]	9.35	13.98[a]	5.02
Antibody-forming cells/10^6 total cells	1790	2262	389	371	258	210	363	365
Total antibody-forming cells (10^3 cells)	8.02	10.85	1.89	2.16	5.18	4.07	4.72	3.92
Responses in Serum								
IgM antibody level (optical density units)	0.71	0.45	0.59	0.43	0.28	0.21	0.56	0.32

[a] Difference from corresponding control group significant at $p < 0.05$ by multiple comparison of log-transformed values.

Table J.2. Significant Influences of Diesel Exhaust and Emphysema on Immune Responses

	Significant Single Influences		Significant Interaction
Parameter	Emphysema	Diesel Exhaust	Emphysema + Diesel Exhaust
Total cells		+	
Antibody-forming cells/10^6 total cells			
Total antibody-forming cells			
Serum IgM			

ABOUT THE AUTHORS

Joe L. Mauderly, the principal investigator of this project, received a doctorate in veterinary medicine from Kansas State University in 1967 and served as a laboratory animal veterinarian and physiologist for the U.S. Air Force at the U.S. Army Natick Laboratories, Natick, MA. He is currently President of the Lovelace Biomedical and Environmental Research Institute and Director of the Inhalation Toxicology Research Institute. Dr. Mauderly's primary research interests are the effects of inhaled toxic materials on the structure and function of the lung, relationships between lung lesions and respiratory functional changes, pulmonary carcinogenesis from inhaled particles, and the extrapolation of toxicological findings in laboratory animals to estimates of health risk for humans.

David E. Bice received a Ph.D. in tropical medicine from Louisiana State University Medical Center in 1968, and performed postdoctoral research at Harvard Medical School and Tufts University School of Medicine. He is currently an immunologist at the Inhalation Toxicology Research Institute, where his research interests include the alteration of lung defense mechanisms by inhaled pollutants and mechanisms responsible for the development of immunity after lung immunization of various animal species.

Yung-Sung Cheng received a Ph.D. in chemical engineering from Syracuse University, Syracuse, NY, in 1976, and performed postdoctoral research at the University of Rochester School of Medicine, Rochester, NY. He is currently an aerosol scientist at the Inhalation Toxicology Research Institute, where he is the Chronic Exposure Section Supervisor. Dr. Cheng's research interests include air sampling for industrial hygiene purposes, inhalation toxicology, deposition in the respiratory tract, fundamental aerosol research, and measurement of radon and radon progeny.

Nancy A. Gillett was awarded a doctorate in veterinary medicine from Washington State University in 1978 and a Ph.D. in comparative pathology from the University of California at Davis in 1984. She is presently an experimental pathologist at the Inhalation Toxicology Research Institute, where she is the Pathophysiology Group Supervisor. Her research emphasizes morphometric analysis of pathology induced by inhaled toxicants and the identification of oncogene expression in neoplastic and preneoplastic lung lesions.

Rogene F. Henderson was a Fulbright Scholar in physical chemistry (Munich), earned a Ph.D. in chemistry at the University of Texas in 1960, and performed postdoctoral research at the University of Arkansas School of Medicine. She is presently a biochemical toxicologist at the Inhalation Toxicology Research Institute, where she is the Chemistry and Biochemical Toxicology Group Supervisor; she also has served as a member of the NIH Toxicology Study Section and is a member of the NAS/NRC Committee on Toxicology. Dr. Henderson's research interests include the biochemistry of the surfactant lining layer of the lung and the development of lavage techniques as a probe to detect lung injury.

John A. Pickrell received a doctorate in veterinary medicine (1965) and a Ph.D. in veterinary medicine science (1968) at the University of Illinois. He is presently an Associate Professor in the Comparative Toxicology Laboratories, Department of Surgery and Medicine, College of Veterinary Medicine, Kansas State University, Manhattan, KS. His research interests include the effects of inhaled toxic materials on lung collagen and elastin and changes in these connective tissue components in various lung diseases.

Ronald K. Wolff earned a Ph.D. in medical biophysics at the University of Toronto in 1972, and is currently Director of the Inhalation Group at Lilly Research Laboratories, Greenfield Laboratories, Greenfield, IN. His research centers on the uptake and distribution of inhaled materials and on the effects of inhaled toxicants on the clearance of particles from the lung.

PUBLICATIONS RESULTING FROM THIS RESEARCH

Mauderly JL, Bice DE, Cheng YS, Gillett NA, Henderson RF, Pickrell JA, Wolff RK. 1987. Influence of emphysema on effects of chronically inhaled diesel exhaust or nitrogen dioxide. Am Rev Respir Dis 135:A284.

Mauderly JL, Cheng YS, Gillett NA, Griffith WC, Henderson RF, Pickrell JA, Wolff RK. 1989. Influence of preexisting pulmonary emphysema on susceptibility of rats to chronic inhalation exposure to nitrogen dioxide. Inhalation Toxicol (in press).

ABBREVIATIONS

ANOVA	analysis of variance
BW	body weight
Cdyn	dynamic lung compliance
CO	carbon monoxide
Cqs	quasistatic chord compliance
^{134}Cs	cesium-134
DL_{CO}	CO diffusing capacity
F_{10}	expiratory flow at 10 percent of forced vital capacity
F_{25}	expiratory flow at 25 percent of forced vital capacity
F_{50}	expiratory flow at 50 percent of forced vital capacity
^{59}Fe	iron-59
FRC	functional residual capacity
FVC	forced vital capacity
HEPA filter	high-efficiency particulate air filter
LV	external lung volume
LW	lung weight
MMEF	mean midexpiratory flow
MV	minute volume
N_2	nitrogen
NO_2	nitrogen dioxide
PEFR	peak expiratory flow rate
$^{239}PuO_2$	plutonium dioxide-239
RV	residual volume
TLC	total lung capacity
VC	vital capacity

EXPERIMENTAL GROUPS

C	untreated, sham-exposed, normal control rats
E	elastase-treated, sham-exposed, emphysematous control rats
N	untreated, NO_2-exposed rats
E + N	elastase-treated, emphysematous, NO_2-exposed rats
D	untreated, diesel-exhaust-exposed rats
E + D	elastase-treated, emphysematous, diesel-exhaust-exposed rats

INTRODUCTION

In the summer of 1982, the Health Effects Institute (HEI) issued a Request for Applications (RFA 82-3) soliciting proposals for "Models of Susceptible Populations." In response to this RFA, Joe L. Mauderly of the Inhalation Toxicology Research Institute, Albuquerque, NM, submitted a proposal entitled "Influence of Preexisting Pulmonary Emphysema on Health Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust." The HEI approved the three-year project, which began in January 1984. Total expenditures were $393,192. The Investigators' Report was received at the HEI in September 1987 and accepted by the Health Review Committee in April 1988. During the review of the Investigators' Report, the Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Commentary. The Health Review Committee's Commentary is intended to place the Investigators' Report in perspective, as an aid to the sponsors of the HEI and to the public.

THE CLEAN AIR ACT

The Environmental Protection Agency (EPA) sets standards for diesel (and other) emissions under Section 202 of the Clean Air Act, as amended in 1977. Section 202(a)(1) directs the Administrator to "prescribe (and from time to time revise) . . . standards applicable to the emission of any air pollutant from any class or classes of new motor vehicles or new motor vehicle engines, which in his judgment cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare." Section 202(a)(3)(A)(i) specifically directs the Administrator to "prescribe regulations . . . applicable to emissions of carbon monoxide, hydrocarbons, and oxides of nitrogen from classes of heavy-duty vehicles or engines." Section 202(a)(3)(A)(iii) similarly requires regulations applicable to emissions of particulate matter from classes or categories of vehicles.

Under these provisions, the EPA has taken regulatory actions with respect to diesel engines. In 1980, the agency set light-duty diesel particulate standards and, in 1984, granted a two-year delay in their effective date. In 1988, the agency established revised particulate standards for certain light-duty diesel trucks. The EPA established emissions averaging in 1983, and it set nitrogen oxides standards for light-duty diesel engines in 1985. For heavy-duty diesel engines, the agency set hydrocarbon and carbon monoxide standards in 1983, and nitrogen oxides and particulate standards in 1985.

Section 109 of the Clean Air Act specifies, in part, that "national primary ambient air quality standards . . . shall be ambient air quality standards the attainment and maintenance of which in the judgment of the Administrator of EPA, based on such criteria and allowing an adequate margin of safety, are requisite to protect the public health." The legislative history of the Act makes it clear that the EPA is required to consider the health of sensitive subgroups of the population in setting these ambient air quality standards. A report on the legislation by the Senate Committee on Public Works states, "An ambient air quality standard, therefore, should be the maximum permissible ambient air level of an air pollution agent or class of such agents (related to period of time) which will protect the health of any group of the population" (U.S. Senate 1970). The definition of such groups is not entirely clear, although the report does specify that "included among those persons whose health should be protected by the ambient standard are particularly sensitive citizens such as bronchial asthmatics and emphysematics who in the course of daily activity are exposed to the ambient environment."

A study of the effects of nitrogen dioxide and diesel-exhaust exposure in an emphysema model in animals can contribute knowledge useful in making the evaluations of probable health effects in humans that are the essential part of informed regulatory decision making under the Clean Air Act.

BACKGROUND

Exhaust from the engines of motor vehicles is a complex mixture of chemicals in the form of gases and solid and liquid aerosols. These emissions, whether from gasoline- or diesel-powered engines, contribute significantly to the burden of outdoor air pollutants. Carbon monoxide, nitrogen oxides, volatile organic compounds, and suspended particulates constitute the main groups of pollutants originating from transportation sources (Bates and Watson 1988).

The Clean Air Act directs the EPA to establish the National Ambient Air Quality Standards to protect the health of groups of the population that are especially susceptible to the effects of inhaled air pollutants; children, the aged, occupational groups, and individuals with preexisting lung disease, such as asthma and emphysema, are such groups. Some evidence suggests that individuals with

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preexisting pulmonary disease may be more susceptible to inhaled toxins. Incidents of severe air pollution episodes in Donora, PA (Schrenk et al. 1949) and London, England (London Ministry of Health 1954) caused an increase in the reports of morbidity and mortality among the elderly and the sick, suggesting that contaminants in the air were more harmful to individuals with existing pulmonary disease than to healthy individuals. Sweeney and colleagues (1988) have reviewed the susceptibility to pulmonary disease in animals with preexisting disease. Results from several studies indicate that, in the lungs of animals with pulmonary disease, airborne particulates both deposit and are removed in patterns that differ from those in healthy animals. The data are conflicting, but the overall results suggest that the deposition site is determined by the distribution of disease in the lungs and the clearance mechanisms affected are dependent upon the pattern of deposition.

Emphysema is one of the conditions specifically identified in the Clean Air Act, and much current research is focused on humans or animal models of emphysema. Emphysema, along with chronic bronchitis, ranks second only to coronary artery disease as a reason for disability compensation in this country (Hoidal and Niewoehner 1983). Direct intratracheal instillation of elastolytic enzymes into the lungs of animals is frequently used as a model of emphysema. Although the elastase-induced model has its shortcomings, it is widely accepted as the state-of-the-art animal model that is used to study the genesis, morphology, and pathology of human emphysema (Snider et al. 1985).

The goal of the study proposed by J.L. Mauderly was to test the hypothesis that preexisting lung disease increases susceptibility to the adverse effects of subsequent exposures to air pollutants. J.L. Mauderly chose elastase-induced emphysema in rats as the model of preexisting lung disease, and nitrogen dioxide and diesel-engine exhaust as the pollutants.

Although the effects of diesel-engine exhaust and nitrogen dioxide have been widely studied in healthy animals, the effects of long-term exposure to either of these pollutants have not been investigated in emphysematous animals. Mauderly and coworkers (1988) have described the nonneoplastic effects in healthy animals exposed to various levels of diesel-engine exhaust for 30 months, and have reported that the lung burdens of diesel soot at the high levels of exposure were greater than what they had projected on the basis of the low level of exposure; this discrepancy was attributed to an impairment of pulmonary clearance mechanisms. Aggregates of carbonaceous material in the alveolar macrophages and lung-associated lymph nodes, and foci of metaplasia in the alveoli and terminal bronchioles, were also noted in the animals exposed to high levels of diesel-engine exhaust.

In addition to the effects noted above, foci of fibrosis (excess collagen deposits in the lung) have been noted in close proximity to large clusters of macrophages after chronic exposure to diesel-engine exhaust (Wiester et al. 1980; Vostal et al. 1982; Hyde et al. 1985; Mauderly et al. 1988). Exposure to particulates leads to increased recruitment of macrophages and polymorphonuclear leukocytes (Mauderly et al. 1988; Sun et al. 1988), and exposure to particle-associated organic compounds has been shown to cause impaired phagocytosis and altered antibody-forming cells, as well as decreased production of antibody in lung-associated lymph nodes (Bice et al. 1985; Sun et al. 1988).

Experimental studies in animals exposed to 20 parts per million (ppm)[1] or higher concentrations of nitrogen dioxide reveal morphological, physiological, and biochemical changes indicative of small airway destruction that leads to the development of emphysema (Freeman et al. 1968; reviewed by Wright 1988). Exposure to low levels of nitrogen dioxide in animals has been shown to cause injury to the pulmonary epithelium down to the small airways of the lung (Evans 1984). Low levels of nitrogen dioxide have also been shown to cause decreased immunological responses, increased levels of lung proteolytic enzymes and peptides, and hematological alterations (see reviews by U.S. Environmental Protection Agency 1982, 1986; Morrow 1984; Samet et al. 1987; Pennington 1988).

Thus, it is reasonable to postulate that, compared with healthy individuals, individuals with preexisting emphysema may be more susceptible to the effects of diesel-engine exhaust or nitrogen dioxide.

JUSTIFICATION FOR THE STUDY

Responding to the concern that some groups in the population may be unusually responsive to mobile-source emissions, the HEI solicited proposals for studies in animals or humans to identify and characterize such subpopulations. Genetic factors, personal activity patterns, previous injury, preexisting disease, and age were listed as the types of factors that might influence both exposure and response to inhaled pollutants. The use of animal models for vulnerable human populations was deemed particularly relevant.

In response to RFA 82-3, Dr. Mauderly and his colleagues proposed a study to examine whether or not preexisting re-

[1] A list of abbreviations appears at the end of the Investigators' Report for your reference.

spiratory disease increased susceptibility to airborne pollutants. They proposed to expose animals with elastase-induced emphysema to nitrogen dioxide or diesel-engine exhaust for two years and compare them with similarly exposed nonemphysematous animals. The endpoints to be examined included various biochemical, physiological, and morphological measures of pulmonary function and health status; carcinogenic effects were not to be assessed.

The study was expected to contribute to the scientific understanding of the health effects of exhaust emissions in subpopulations with emphysema and to point to new directions for research on the interactions between inhaled pollutants and preexisting lung disease.

GOALS AND OBJECTIVES

The goal of this study was to test the hypothesis that preexisting lung disease increases susceptibility to the toxic effects of inhaled air pollutants. Using a model of chronic pulmonary disease, elastase-induced pulmonary emphysema, emphysematous and healthy rats were exposed to nitrogen dioxide or diesel-engine exhaust, and the effects on pulmonary tissues, deposition and clearance, biochemistry, and respiratory function were compared.

STUDY DESIGN

A total of 276 rats was divided equally into two groups, one of which received intratracheal porcine pancreatic elastase to induce pulmonary emphysema. Six weeks later, 92 rats (46 healthy and 46 with emphysema) were assigned to each of three exposure atmospheres (9.5 ppm nitrogen dioxide; 3,500 μg soot/m^3 diesel-engine exhaust; and sham-exposed controls) for seven hours per day, five days per week, for 24 months. At 12, 18, and 24 months, the effects of the exposures were evaluated by studying respiratory function, pulmonary histopathology and morphology, lung proteases, enzyme and cytologic profiles in bronchoalveolar fluids, and lung burdens of diesel particulates. In addition, the immunologic response of the lung-associated lymph nodes to sheep red blood cells and the clearance of inhaled radiotracer particles were measured at 24 months.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

The investigators have achieved their objectives. Valuable information has been obtained through the completion of the originally established goals.

ASSESSMENT OF METHODS, STUDY DESIGN, AND DATA ANALYSIS

The experimental design was appropriate for evaluating the influence of elastase-induced emphysema on the effects of inhaled nitrogen dioxide and diesel-engine exhaust. Although many parameters of effect were evaluated, the extent to which they are interdependent, or measure the same underlying biological mechanism, is not clear.

The authors, who have extensive experience in carrying out studies of this nature, executed the current investigations well. The exposure levels of 3,500 μg soot/m^3 diesel-engine exhaust and 9.5 ppm nitrogen dioxide were higher than the typical levels of human exposure; therefore, findings cannot be directly extrapolated to human populations. However, these levels were chosen by the investigators because health effects had been observed previously in their laboratory at these levels. Exposure levels that ensure the induction of health effects were deemed necessary to compare the effects of exposure in normal and emphysematous animals.

For comparing the effects of exposure to nitrogen dioxide or diesel-engine exhaust in emphysematous animals with those in nonemphysematous animals, analysis of variance, which was appropriate for the study, was used.

Multiple parameters were measured at six-month intervals over a period of two years. The statistical analyses addressed three different types of interactions. First, all the parameters were examined to detect statistically significant differences between treated animals (emphysema [E] or pollutant exposure) and control [C] animals. Second, the group with both emphysema and pollutant exposure (either nitrogen dioxide [E + N] or diesel-engine exhaust [E + D]) was compared to the group with pollutant exposure alone (either nitrogen dioxide [N] or diesel-engine exhaust [D]) to assess whether or not differences in response existed that could be attributable to the preexisting disease. Third, the directions and magnitudes of the differences between each of the comparisons were examined to determine if the influence of emphysema and pollutant exposure was additive, more-than-additive, or less-than-additive.

As the investigators described in the Methods section, "additive" was defined to mean that the sum of the differences due to emphysema or exposure alone were the same as the difference due to the combined treatment; that is:

$$(E - C) + (N - C) = [E+N] - C$$

or

$$(E - C) + (D - C) = [E+D] - C.$$

Therefore, if the right-hand term was found to be greater than the sum of the left-hand differences, the effect would be judged to be more-than-additive, and if it was less, the effect would be less-than-additive. (It must be kept in mind that the individual terms of these relationships cannot be subjected to algebraic manipulation.) Findings of more-than-additive parameters would be an indication that rats with pulmonary emphysema were more susceptible than rats with healthy lungs to the adverse effects of exposure to nitrogen dioxide or diesel-engine exhaust.

INTERPRETATION OF RESULTS

The emphysematous condition was well developed and well documented. The intratracheal elastase treatment resulted in stable pulmonary emphysema, as manifested by enlarged alveoli and alveolar ducts, and by ruptured alveolar septa, without alteration of bronchioles or persistent inflammation. The emphysema produced an increase in lung weight, enlargement of physiologic lung volumes, a decrease in lung density, an increase in lung compliance, and airflow obstruction.

Chronic diesel-engine exhaust exposure in normal rats produced progressive focal inflammation, epithelial proliferation, and fibrosis surrounding foci of aggregated soot-laden macrophages in alveoli, with the lungs becoming smaller, stiffer, and heavier. Airway fluid showed signs of increased cytotoxicity, proteolytic activity, and turnover of lung collagen. Pulmonary lymph nodes showed increased cellularity, but no alterations in response to antigen challenge (sheep red blood cells).

In agreement with earlier chronic studies (Evans 1984; Wright 1988), nitrogen dioxide exposure in normal rats caused a mild epithelial hyperplasia, with thickening of the walls of the terminal bronchiole and extension of the bronchiolar epithelium into the proximal alveoli. Lung volume and lung weight were increased, as was the collagen content of the lung. There was histological evidence of inflammation in the proximal alveoli, as well as the indication of cell damage and of stimulation of the oxidant-protective (glutathione) pathway in the bronchioalveolar lavage studies.

In emphysematous animals exposed to diesel-engine exhaust, the accumulation of soot was reduced, the final lung burden being only one-third as large as that found in the nonemphysematous animals. The diesel particulates were observed to deposit more centrally and to be cleared faster than in control animals. While the effects of diesel-exhaust exposure in emphysematous rats were similar to those in nonemphysematous rats, the magnitude of the effects was reduced. Significant interactions between emphysema and exposure were observed in 18 parameters (out of 65 parameters measured) relating to respiratory function, airway fluid, particle clearance, lung collagen content, and lung weight; all parameters varied similarly in being less-than-additive. Thus, the lung structure and function of the emphysematous animals exposed to diesel-engine exhaust appeared to be compromised more than those of emphysematous animals that had not been exposed to the pollutant, but less than those of nonemphysematous animals exposed to diesel-engine exhaust; these differences are attributed to the lower levels of diesel soot deposited in the lungs of the emphysematous rats.

In the emphysematous animals exposed to nitrogen dioxide, the effects observed were qualitatively similar to those noted in the nonemphysematous animals. No interaction was observed in 57 out of the 61 parameters that were measured. Regarding the remaining four parameters, the effects on expiratory flow at 10 percent of forced vital capacity and on cathepsin B levels were additive; the effect on pulmonary flow at 25 percent of forced vital capacity was more-than-additive; and the effect on acid proteinase was less-than-additive. (Note that if all 61 parameters were independent, one would expect three significant effects to be measured by chance alone at the $p = 0.05$ level.) The authors' overall conclusion was that there was no interaction between the two treatments. However, because nitrogen dioxide and emphysema caused most parameters to be influenced in the same direction, emphysematous animals were interpreted to be more affected by, and therefore at greater risk from, nitrogen dioxide exposure than were normal animals.

The authors' conclusion is reasonable, but the relation of the various measures to one another and to the overall health outcome remains uncertain. The parameters cannot necessarily be compared on a linear scale. In addition, the elastase-induced emphysema model itself introduces difficulty in interpretation. For example, both acid proteinase and cathepsin B are measures of proteolytic activity. However, because the animals had been subjected to an extensive proteolytic treatment at the beginning of the study, it is difficult to predict whether any changes in measures of proteolytic activity observed in emphysematous rats subsequently exposed to nitrogen dioxide would be the result of exposure to nitrogen dioxide or the result of induced emphysema.

The authors' original hypothesis was that the exposure-related effects in emphysematous rats would be greater-than-additive. This hypothesis should not lead one to conclude that findings of less-than-additive effects between emphysema and the pollutant are unimportant. The authors have explored these interactions in two ways: They attempted to ascertain (1) whether or not rats with emphysema

have more combined adverse health effects when exposed to pollutant than do rats without emphysema (the relevant question for human populations); and (2) whether the effects of emphysema and pollutant are less-than-additive or more-than-additive. It is noteworthy that in general the combined effects of emphysema and exposure to a pollutant were more severe than exposure to a pollutant alone.

The authors concluded that stable elastase-induced emphysema did not increase lung susceptibility to subsequent exposure to nitrogen dioxide or diesel-engine exhaust. The increased changes produced by pollutant exposure combined with emphysema resulted from the effects of exposure superimposed on the effects of the underlying disease. More-than-additive adverse interactive health effects (synergism), such as would have been associated with hypersusceptibility, were not observed.

These findings have an important bearing on qualitative concepts of risk assessment—that is, the effects of prior disease on susceptibility to pollutant exposure. The finding that the effects were not more-than-additive is important for assessing quantitatively the levels of exposure to pollutants that may be harmful to human health.

FUTURE RESEARCH NEEDS

One of the major uncertainties that remains is the biological interpretation of the parameters measured for detection of adverse health effects. The measures chosen—respiratory function, particle clearance, immune responses, bronchoalveolar lavage analyses, lung collagen content, lung histology and morphometry, and particle burden, among other possible assays and measurements—are those that the authors believed to be the most directly relevant to the evaluation of the effects of pollutant exposure on emphysema in rats. Establishment of a hierarchy among the different types of assays, and among the different parameters within assays, is needed to clarify the relevance of the changes for pulmonary health, but cannot be achieved without expansion of the data base and further analysis of the interrelationships among the measures. In addition, future studies designed to explore the mechanisms of emphysema and the effects of nitrogen dioxide and diesel-exhaust exposure will contribute to the interpretation of the multiple parameters of response that are well documented in this study.

In elastase-treated animals, inhaled diesel particles deposited more centrally and were cleared faster than in control animals, but the investigators did not study the reasons for the different deposition patterns. To do so would have required measurement of airway flow patterns and other parameters that were beyond the scope of this study. Nevertheless, the results of this study have increased our understanding of particle deposition in emphysematous rats. The results also indicate that the question of the effect of emphysema on particle clearance is only partially answered and deserves further investigation. Additional information is needed to predict fractional and regional deposition in other species, including humans.

A general research direction arising from the current study is the need to develop and refine animal models for the bronchiolitis and centriacinar lesions associated with chronic obstructive pulmonary disease, in order to improve quantitative extrapolations of the combined effects of preexisting disease and pollutant exposure.

CONCLUSIONS

This study has provided evidence that rats with experimentally induced emphysema show more dysfunction from inhalation exposure to air pollutants than normal rats because their lungs are already compromised by underlying disease. On the other hand, as these combined effects are not more-than-additive, this study has demonstrated that experimental emphysema does not create a condition of higher susceptibility to pollutant exposure (nitrogen dioxide or diesel-engine exhaust). Nevertheless, the study confirms the need to concentrate on emphysematous subpopulations in risk estimation and standard setting because the effects of the underlying disease may add to the effects of the air pollutant.

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LIST OF HEI PUBLICATIONS

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Special Reports

Title	Publication Date
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research	September 1985
Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research	May 1987
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research (Supplement)	January 1988

Research Reports

Report No.	Title	Principal Investigator	Publication Date
1	Estimation of Risk of Glucose 6-Phosphate Dehydrogenase-Deficient Red Cells to Ozone and Nitrogen Dioxide	M. Amoruso	August 1985
2	Disposition and Metabolism of Free and Particle-Associated Nitropyrenes After Inhalation	J. Bond	February 1986
3	Transport of Macromolecules and Particles at Target Sites for Deposition of Air Pollutants	T. Crocker	February 1986
4	The Metabolic Activation and DNA Adducts of Dinitropyrenes	F.A. Beland	August 1986
5	An Investigation into the Effect of a Ceramic Particle Trap on the Chemical Mutagens in Diesel Exhaust	S.T. Bagley	January 1987
6	Effect of Nitrogen Dioxide, Ozone, and Peroxyacetyl Nitrate on Metabolic and Pulmonary Function	D.M. Drechsler-Parks	April 1987
7	DNA Adducts of Nitropyrene Detected by Specific Antibodies	J.D. Groopman	April 1987
8	Effects of Inhaled Nitrogen Dioxide and Diesel Exhaust on Developing Lung	J.L. Mauderly	May 1987
9	Biochemical and Metabolic Response to Nitrogen Dioxide-Induced Endothelial Injury	J.M. Patel	June 1987
10	Predictive Models for Deposition of Inhaled Diesel Exhaust Particles in Humans and Laboratory Species	C.P. Yu	July 1987
11	Effects of Ozone and Nitrogen Dioxide on Human Lung Proteinase Inhibitors	D.A. Johnson	August 1987
12	Neurotoxicity of Prenatal Carbon Monoxide Exposure	L.D. Fechter	September 1987
13	Effects of Nitrogen Dioxide on Alveolar Epithelial Barrier Properties	E.D. Crandall	October 1987
14	The Effects of Ozone and Nitrogen Dioxide on Lung Function in Healthy and Asthmatic Adolescents	J.Q. Koenig	January 1988
15	Susceptibility to Virus Infection with Exposure to Nitrogen Dioxide	T.J. Kulle	January 1988
16	Metabolism and Biological Effects of Nitropyrene and Related Compounds	C.M. King	February 1988

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Research Reports

Report No.	Title	Principal Investigator	Publication Date
17	Studies on the Metabolism and Biological Effects of Nitropyrene and Related Nitro-polycyclic Aromatic Compounds in Diploid Human Fibroblasts	V.M. Maher	March 1988
18	Respiratory Infections in Coal Miners Exposed to Nitrogen Oxides	M. Jacobsen	July 1988
19	Factors Affecting Possible Carcinogenecity of Inhaled Nitropyrene Aerosols	R.K. Wolff	August 1988
20	Modulation of Pulmonary Defense Mechanisms Against Viral and Bacterial Infections by Acute Exposures to Nitrogen Dioxide	G.J. Jakab	October 1988
21	Maximal Aerobic Capacity at Several Ambient Concentrations of Carbon Monoxide at Several Altitudes	S.M. Horvath	December 1988
22	Detection of Paracrine Factors in Oxidant Lung Injury	A.K. Tanswell	February 1989
23	Responses of Susceptible Subpopulations to Nitrogen Dioxide	P.E. Morrow	February 1989
24	Altered Susceptibility to Viral Respiratory Infection During Short-Term Exposure to Nitrogen Dioxide	R.M. Rose	March 1989
25	Acute Effects of Carbon Monoxide Exposure on Individuals with Coronary Artery Disease	HEI Multicenter CO Study Team	To be released soon
26	Investigation of a Potential Cotumorigenic Effect of the Dioxides of Nitrogen and Sulfur, and of Diesel-Engine Exhaust, on the Respiratory Tract of Syrian Golden Hamsters	U. Heinrich	May 1989
27	Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure	J.J. McGrath	July 1989
28	Nitrogen Dioxide and Respiratory Infection: Pilot Investigations	J.M. Samet	September 1989
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THE HEALTH EFFECTS INSTITUTE: AN OVERVIEW

HEI

The Health Effects Institute (HEI) is an independent nonprofit corporation that is "organized and operated . . . to conduct, or support the conduct of, and to evaluate research and testing relating to the health effects of emissions from motor vehicles." It is organized in the following ways to pursue this purpose.

INDEPENDENCE IN GOVERNANCE

The Institute is governed by a four-member Board of Directors whose members are Archibald Cox (Chairman of the Board), Carl M. Loeb University Professor (Emeritus) at Harvard University; William O. Baker, Chairman (Emeritus) of Bell Laboratories and Chairman of the Board of Rockefeller University; and Donald Kennedy, President of Stanford University.

TWO-SECTOR FINANCIAL SUPPORT

The Institute receives half of its funds from the United States government through the Environmental Protection Agency, and half from the automotive industry. Twenty-seven domestic and foreign manufacturers of vehicles or engines contribute to the Institute's budget in shares proportionate to the number of vehicles or engines that they sell.

THE HEI RESEARCH PROCESS

The Institute is structured to define, select, support, and review research that is aimed at investigating the possible health effects of mobile source emissions. Its research program is developed by the Health Research Committee, a multidisciplinary group of scientists knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from HEI's sponsors and from other sources prior to independently determining the research priorities of the Institute.

After the Health Research Committee has defined an area of inquiry, the Institute announces to the scientific community that research proposals are being solicited on a specific topic. Applications are reviewed first for scientific quality by an appropriate expert panel. Then they are reviewed by the Health Research Committee both for quality and for relevance to HEI's mission-oriented research program. Studies recommended by the Committee undergo final evaluation by the Board of Directors, who review the merits of the study as well as the procedures, independence, and quality of the selection process.

THE HEI REVIEW PROCESS

When a study is completed, a final report authored by the investigator(s) is reviewed by the Health Review Committee. The Health Review Committee has no role either in the review of applications or in the selection of projects and investigators for funding. Members are also expert scientists representing a broad range of experience in environmental health sciences. The Committee assesses the scientific quality of each study and evaluates its contribution to unresolved scientific questions.

Each Investigator's Report is peer-reviewed, generally by a biostatistician and three outside, independent reviewers chosen by the Review Committee. At one of its regularly scheduled meetings, the Review Committee discusses the Investigator's Report. The comments of the Committee and the peer reviewers are sent to the investigator, and he or she is asked to respond to those comments and, if necessary, revise the report. The Review Committee then prepares its Commentary, which includes a general background on the study, a technical evaluation of the work, a discussion of the remaining uncertainties and areas for future research, and implications of the findings for public health. After evaluation by the HEI Board of Directors, the HEI Research Report, which includes the Investigator's Report and the Review Committee's Commentary, is published in monograph form. The Research Reports are made available to the sponsors, the public, and many scientific and medical libraries, and are registered with NTIS.

All HEI investigators are urged to publish the results of their work in the peer-reviewed literature. The timing of the release of an HEI Research Report is tailored to ensure that it does not interfere with the journal publication process.